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Assessment & design of perfused microalgal biofilm cultivation processes

Thesis submitted by

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I

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Intellectual contributions

- Ross Barett and Curtis Arrowsmith, James Cook University, contributed technical experience for the construction of the biofilm reactors in Chapter 3.
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Abstract

Microalgae are emerging as a promising technology for photosynthetic recycling of carbon dioxide (CO₂) into industrially useable biomass. However, the efficiency of current suspension cultivation systems is limited by low biomass concentration and high dewatering costs. In this thesis I investigated perfused microalgal biofilms as an alternative cultivation process. I designed and built a prototype cultivation system and process, developed a laboratory method for assessing basic cultivation parameters and synthesised the knowledge gained into a mathematical simulation of productivity and power costs of microalgal biofilm cultivation for the production of concentrated feeds in aquaculture.

Microalgae (including cyanobacteria) are a diverse group of microscopic aquatic organisms, many of them capable of photosynthesis. These traits allow the cultivation of microalgae in intensive bio-processes, independent of arable land, using light as an energy source to convert CO₂ into biomass. This makes microalgae potential candidates for mitigating globally increasing CO₂ levels and allows for promising synergies with waste water remediation. The microalgal biomass can be used as feedstock for a wide range of applications, such as biofuels, animal feeds in agriculture and particularly aquaculture and as raw material for green chemistry and pharmaceutical processes. Yet, despite this potential, microalgal cultivation has struggled to gain widespread industrial use. A key challenge is the low cell concentration in current suspension systems, which results in high water requirements and dewatering costs that are economically prohibitive.

Biofilm-based cultivation can provide an answer to this challenge, by growing microalgae at high cell concentration attached to surfaces. This approach promises increased light availability, higher gas exchange rates and overall lower water requirements and substantially reduced dewatering costs. In Chapter 2, I reviewed existing microalgae biofilm cultivation systems and classified them into three distinct groups based on the interaction between surface and cultivation medium: *Constantly submerged systems*, where the biomass is always covered by a thin film of liquid, *intermittently submerged systems*, where the biofilms move in and out of the liquid

IV

phase and *perfused systems* where the biofilm is directly exposed to the surrounding atmosphere.

A system of this last group was the focus of Chapter 3, which describes the design and development of a perfused membrane photo-biofilm reactor and its assessment under tropical greenhouse conditions. A biofilm of *Mesotaenium sp.* was successfully cultivated and growth curves were obtained, showing a maximal biomass productivity of up to 1.7 g m⁻² d⁻¹ (dry weight) and a maximal biomass yield of 21.25 g m⁻² (dry weight). Spatial variations in growth were correlated with high temperatures (above 39 °C) and a corresponding drop in relative humidity which led to dehydration of part of the biofilms. This represents a new finding not previously described in perfused biofilm cultivation in temperate conditions and will influence the design of future cultivation processes, especially in the tropics.

The findings from the literature and the experience with the prototype also highlighted the need for simpler test methods to allow for standardised testing of basic cultivation parameters under reproducible laboratory conditions. In Chapter 4, a petri-dish assay was developed which allows the cultivation of perfused biofilms on different materials at low cost and technical requirements. The use of this assay was successfully demonstrated by investigating the growth behaviour of different microalgal species, as well as growth on different surface materials and under different light conditions. This assay was also used in a related Honours thesis project, which showed the potential use of *Isochrysis* aff. *glabana* biofilms as concentrated aquaculture feed and replacement for commercial algal paste.

The results of these investigations were applied in Chapter 5. The growing aquaculture industry has led to an increasing demand for high-quality microalgal concentrates. Cultivation of microalgae in suspension and biofilm cultivation systems was simulated, at the scale of an aquaculture hatchery, in order to compare productivity and power costs in batch and semi-continuous growth modes. Biofilm cultivation was shown to be feasible at similar scale to suspension cultivation, but still in need of improvement to be competitive. This simulation could be further expanded into a techno-economic analysis by incorporating data from existing industrial processes.

V

The overarching aim of this thesis was to investigate and develop new approaches for microalgae cultivation in order to open up new, sustainable industrial production pathways. This was achieved by assessing a rapidly developing field, identifying the potential of perfused biofilm technology and by designing cultivation processes and systems based this approach. The final productivity and power simulation relates the knowledge gained to a promising industrial application with short and long term potential in Australia and worldwide. In a broader context this work provides a summary off the current state of applied microalgal biofilm research, highlights the importance of experiments under relevant environmental conditions, and provides analytical and computational research tools for improved assessment of applied microalgal biofilm cultivation.

Table of contents

Acknowledgements	I
Statement of the Contribution of Others	
Abstract	IV
Table of contents	IV
List of tables	VII
List of figures	VIII
Chapter 1: General Introduction	1
1.1 Photosynthetic microalgae production	1
1.2 Challenges	2
1.3 Biofilm based production	3
1.4 Chapter structure	4
Chapter 2: Microalgal biofilms for biomass production	6
2.1 Abstract	7
2.2 Introduction	8
2.3 Biofilm development in a production context	10
2.4 Biofilm-based cultivation systems for microalgal biomass	production12
2.4 The need for standardisation	27
2.5 Conclusions	29

cu	Iltivation in tropical conditions	31
	3.1 Abstract	32
	3.2 Introduction	33
	3.2 Design and construction of the perfused membrane biofilm reactor	35
	3.3 Material and Methods	39
	3.4 Results	41
	3.5 Discussion	45
	3.6 Conclusions and recommendations	47
	3.7 Acknowledgements	48
Cł	napter 4: Petri-dish assays as a research tool for perfused microalgal	
bi	ofilms	49
	4.1 Abstract	49
	4.2 Introduction	50
	4.3 Test scenarios	55
	4.4 Materials & Methods	58
	4.5 Results	60
	4.6 Discussion:	66
	4.7 Conclusion	68

Chapter 3: A perfused membrane biofilm reactor for microalgae

Chapter 5: Simulation of biofilm and suspension cultivation processes for
the production of microalgal concentrates in aquaculture
5.1 Abstract69
5.2 Introduction71
5.3 Scope definition74
5.4 Numerical approach75
5.5 Model description76
5.5 Evaluation & Results95
5.6 Conclusions103
5.7 Acknowledgements105
Chapter 6: General discussion 106
6.1 Summary106
6.2 Main conclusions and outcomes106
6.2 Outlook112
6.3 Overall conclusion118
References
Appendix 3.1: Provisional patent application136
Appendix 5.1 Variables for simulation172
Appendix 5.2: Microalgal growth methods175
Appendix 5.3: Power use 177
Appendix 5.4: MATLAB code 180

List of tables

Table 2.1	Constantly submerged systems	14
Table 2.2:	Intermittently submerged systems	16
Table 2.3:	Perfused systems	20
Table 3.1:	Average biomass production in a PMBR	42
Table 4.1:	Comparison of two different cultivation systems	53
Table 4.2:	Specific growth rates of suspension cultures	61
Table 4.3:	Amount of biomass and filters required for biochemical profiling	67
Table 5.1:	Specific growth rates for biofilm cultivation	92
Table 5.2:	Number of bags required for semi-continuous suspension cultivation	98
Table 5.3:	Number of panels required for semi-continuous biofilm cultivation	100
Table 5.4:	Comparison of power consumption	102
Table 5.5:	Biofilm area to match bag production	104
Table 6.1:	R_L for different number of panels in an RPM-PBR	110

List of figures

Figure 3.1:	Operating principle of perfused membrane biofilm reactors	34
Figure 3.2:	Details of the vertical cultivation panel	36
Figure 3.3:	Rotating perfused membrane biofilm reactor	38
Figure 3.4:	Temperature and relative humidity during the vertical panel cultivation of <i>Mesotaenium sp</i> . biofilms.	41
Figure 3.5:	Growth curve of. in a single panel PMBR	42
Figure 3.6:	Filters on the east side of the panel of the PMBR with. biofilms on day 21	43
Figure 3.7:	Deviation of biomass of <i>Mesotaenium sp.</i> from average for the six different zones of the vertical PMBR.	44
Figure 4.1:	Overview of the petri-dish assay	55
Figure 4.2	Growth curves for biofilms of four different species.	61
Figure 4.3	Relative biomass growth of biofilm and suspension cultures	62
Figure 4.4:	Growth of <i>Mesotaenium sp.</i> biofilm under indoor and outdoor conditions.	63
Figure 4.5:	Growth of <i>Mesotaenium, Isochrysis</i> aff. <i>galbana</i> and <i>Prymnesium</i> on different substrate materials.	64
Figure 4.6:	Correlation between cell counts m ⁻² and dry weight m ⁻²	65
Figure 5.1:	Overall process flow for microalgal cultivation, showing the 4 processing steps.	75
Figure 5.2:	Schematic overview of the microalgal bag cultivation system.	78

Figure 5.3:	Growth curve of Isochrysis aff. galbana (TISO, NQAIF 001)	79
	suspension cultures in f/2 (Guillard and Ryther 1962;	
	Guillard 1975).	

- Figure 5.4:Results of the baseline batch cultivation process84
- Figure 5.5Microalgal biomass concentrations in a semi-continuous86bag cultivation process, $(T_{BATCHSCB} = 12 d and, T_{RGSCB} = 8 d)$ versus the experimental growth data.
- Figure 5.6:Simulation of the microalgal biomass concentration in the87semi-continuous microalgal bag cultivation process, for $T_{BATCHSCB} = 12 d and, T_{RGSCB} = 4 d.$
- Figure 5.7: Schematic overview of the PMBR panel cultivation system 89
- Figure 5.8: Operating principle of perfused membrane photo-biofilm 90 reactors.
- Figure 5.9: Growth curve of *Isochrysis aff. galbana* (TISO) biofilms on 91 different materials
- Figure 5.10: Number of PMBR panels needed for microalgal biofilm 99 batch cultivation
- Figure 6.1:Illustration of the light dilution factor (R_L) in a 6 panel109RPM-PBR.
- Figure 6.2: Overview of mass transfers in a perfused photo-biofilm 116 reactor

Chapter 1: General Introduction

The overarching theme of this thesis is the improvement of industrial photosynthetic microalgae production processes by applying biofilm cultivation techniques. This combines two emerging and rapidly developing fields of research, both rich with opportunities, but also with no shortage of challenges.

This general introduction places this thesis into the general context of this research, and introduces the chapter structure. As each chapter of this thesis is intended as a standalone publication, more detailed introduction to the specific aspects investigated is presented at the beginning of each chapter.

1.1 Photosynthetic microalgae production

This thesis was originally conceived as part of the JCU/AMCRC/MBD Energy research cooperation, a project with the ambitious goal to use microalgae to remediate carbon dioxide (CO₂) from coal fired power stations by producing biodiesel.

Microalgae (including cyanobacteria) are a highly diverse group of microorganisms, spanning several taxonomic groups and include both prokaryotes (cyanobacteria) and eukaryotes (several different phyla). While the ability for photosynthesis is not universal, it is the defining feature for the group with regards to most industrial applications (Grobbelaar 2012), as this enables the biological conversion of CO₂ into biomass, using light to provide the necessary energy for this process. (Stephens et al. 2010; Ho et al. 2011; Milledge 2011; Satyanarayana et al. 2011). As such, this technology resonates well with the current global challenges of climate change due to levels of CO₂ production (including by coal fired power stations) and with future resource uncertainty in view of a globally increasing population (Ho et al. 2011; IPCC 2014).

As microorganisms, microalgae are especially suited for intensive industrial bioprocesses. Individual cells have a large surface to volume ratio and short diffusion lengths between the outside of the cell and reaction sites within. This allows for rapid exchange of molecules with the environment, rapid metabolism and consequently very rapid growth. Furthermore, microalgal cultivation systems are independent of soil

quality and offer a high degree of flexibility with regards to size, shape and location of the cultivation systems (Schenk et al. 2008; Singh et al. 2011). In addition, there are well established synergies between microalgae cultivation and wastewater treatment, allowing to add further value to a remediation process (Hoffmann 1998; Munoz et al. 2009; Markou and Georgakakis 2011).

Microalgal biomass can be used in a wide variety of applications, the largest of which – both in terms of potential market and amount of ongoing research – is the production of biofuels. Some species of microalgae can achieve high lipid contents, under the right environmental and nutrient conditions (Chisti 2007; Benemann 2013). This increases the caloric value of the biomass and allows for the production of biofuels, such as biodiesel (by lipid extraction and transesterification), syngas or bio-oil (by thermochemical conversion, such as gasification or hydrothermal liquefaction), or direct combustion of the biomass to replace other fuel sources (Brennan and Owende 2010).

As such, at face value, there seems to be considerable economic potential in an industrial photosynthetic microalgae production process, using CO₂ from a coal fired power station (Benemann 1997; Stepan et al. 2002; Mata et al. 2010).

1.2 Challenges

However, there are still open questions regarding the economic feasibility of biofuels, a low value, bulk product which requires large-scale cultivation facilities with significant initial investments (Klein-Marcuschamer et al. 2013; Pate 2013; Ribeiro and Silva 2013). Consequently, in recent years there has been a shift away from biofuels towards products that can be implemented at smaller scale, for higher value products. This includes pigments and anti-oxidants, human and animal feeds and precursors for green chemistry (Milledge 2011; Clark et al. 2012; Borowitzka 2013a).

These questions were also being assessed as part of the JCU/AMCRC/MBD Energy research cooperation. One of the key challenges identified, that led directly to this thesis, was the low biomass concentration in large scale cultivation systems and its consequences for the overall process.

The scale of the planned production at a power station would have required low-cost outdoor systems which have reported biomass concentrations around 0.5 g (dry weight, DW) $|^{-1}$ (Lee 2001), equal to 0.05 % total suspended solids (discussed in more detail in the introduction for Chapter 2). From another perspective, the production of 1 kg of dry microalgal biomass requires processing of 2000 I (or kg) of cultivation medium. This low biomass to medium ratio increases the cost throughout the production process, from preparation of the medium, to moving and mixing these volumes in the cultivation systems and to the eventual harvesting and dewatering to produce concentrated biomass (Molina Grima et al. 2003; Uduman et al. 2010). There is ongoing research into improvement off all of these aspects in suspension cultivation processes, but in this thesis I decided to follow a novel approach, by investigating a fundamentally different way of cultivating microalgae, attached to a surface, rather than as free swimming cells – the biofilms.

1.3 Biofilm based production

Biofilms are defined by the international union of pure and applied chemistry (IUPAC) as "Aggregate of microorganisms in which cells, that are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS), adhere to each other and/or to a surface." (Vert et al. 2012). This is not in itself a radical realisation, as this adherence has long been observed in many natural systems, in nature, the human body and industry (Mattila-Sandholm and Wirtanen 1992; Costerton et al. 1995; Hall-Stoodley et al. 2004). Microalgal biofilms are a recognised and well-studied part of the marine and freshwater benthos and the so called microphytobenthos provides a number of important ecological functions, such as primary production, food source for a wide range of organisms and stabilisation of sediments (Cooksey and Wigglesworth-Cooksey 1995; Miller et al. 1996; Mieszkin et al. 2013). In industrial systems, biofilms have long been used for wastewater treatment (Lazarova and Manem 1995; Nicolella et al. 2000; Adey et al. 2011; Orandi and Lewis 2013), but are also frequently encountered as undesired biofouling, that occurs on almost any wet surface with sufficient nutrients (Mattila-Sandholm and Wirtanen 1992; Flemming et al. 1996; Townsin 2003). For their constituent cells, Biofilms have been recognised to provide a range of benefits, such as increased environmental tolerance and resistance to antibiotics and some extent of protection from predation (Costerton et al. 1995; Hall-Stoodley et al. 2004).

From a bio-production perspective, microalgal biofilms are of interest as the cells on the surfaces are growing in close proximity, leading to a high biomass concentrations. Since the cells are attached to the surface, they can adapt to the specific microenvironment and light conditions depending on their location in the biofilm, and can optimise their metabolism for these conditions. Another benefit is that the biofilms only need to be covered by a minimal amount of liquid medium (or even none at all), to maintain moisture and provide nutrients. This reduces overall water requirements and the thin medium layer interferes less with the light penetration to the surface of the biofilm and allows for increased gas exchange rates.

From a process perspective, the surface and the cultivation liquid can easily be moved independently each other, making the biofilms accessible for harvesting without the need for concentration steps. These points are further expanded in Chapter 2, where the specific approaches to microalgal cultivation are reviewed and discussed in detail.

The sum of these benefits leads to the proposition, that biofilms could be an alternative approach to cultivation and led directly to the formulation of the overall aim of this thesis: To develop approaches to quantify and compare industry-scale photosynthetic microalgae production processes using perfused biofilm cultivation.

1.4 Chapter structure

Chapter 2 reviews the state of knowledge regarding microalgal biofilm cultivation for biomass production. This chapter aims to identify the predominant concepts in the field and to identify promising opportunities for future applied research.

Chapter 3 describes the development and testing of prototype cultivation systems, based on the principle of perfused biofilms. This approach was identified during the review for chapter 2 and was identified as especially promising due to the direct exposure of the biofilm to the surrounding environment.

Chapter 4 addresses the need for simpler experimental tests with the development of a laboratory scale Petri-dish assay for perfused biofilms. This test aims to address the lack of standardisation across the field, identified in chapter 2, and to provide a tool to isolate compounding factors affecting biofilm productivity, which were identified during chapter 3.

Chapter 5 builds up on the knowledge and experience gained, by developing a mathematical simulation of productivity and power cost for comparing suspension and biofilm based production of concentrated microalgal feeds in aquaculture - a shift in focus to a higher value product than biofuels, as it has taken occurred in other parts of the field.

Chapter 6 synthesises the key-findings of each chapter and discusses these in the context of research progress made since and future research and development opportunities arising from the presented research outcomes.

Chapter 2: Microalgal biofilms for biomass production

This chapter was published as: "Berner F, Heimann H, Sheehan M (2014) Microalgal biofilms for biomass production; Journal of Applied Phycology, 27: 1793-1804".

The paper was written in its entirety by myself, Kirsten Heimann and Madoc Sheehan provided academic guidance and editorial input.

The work is presented as published, except for the conclusion that has been expanded after comments by examiners. Furthermore, the formatting has been changed to match that of the rest of this thesis.

2.1 Abstract

Microalgae are promising candidates for recycling of carbon dioxide (CO₂) into renewable bioproducts. However, the low biomass concentration of current suspension culture systems leads to high water requirements, inefficient harvesting and high liquid transportation costs. Despite ongoing research, these still propose a challenge to the economic viability of microalgal cultivation.

Microalgal biofilms provide an alternative approach to biomass production that could resolve these challenges, by growing the cells attached to a surface, surrounded by a self-produced matrix of polymers. Microalgal biofilms have much higher biomass concentrations than suspension cultures and the attached cells are easy to separate from the cultivation medium. However, cultivating microalgal biofilms requires the development a purposefully designed cultivation systems, especially due to interactions between cells and surface, persistent gradients in the biomass and the effects of flow, which play a critical role for biofilm productivity.

A range of systems has been employed for the cultivation of microalgal biofilms, with biomass productivities of up to 60 g(DW) m⁻² d⁻¹ (dry weight per ground area) outdoors and up to 80 g(DW) m⁻² d⁻¹ under laboratory conditions, respectively. However, there is considerable variation of reported results along with experimental conditions, which limits the capability for quantitative comparisons with other systems and hinders the identification of the drivers and variables that dictate microalgal biomass formation. Development of standard conditions and representative species would be required for closing this gap and for realising the full potential of microalgal biofilm cultivation as a viable process for industrial biomass production.

2.2 Introduction

The significant potential of microalgae for photosynthetic production of biomass and derived bioproducts has been extensively reviewed recently, covering a wide range of research areas (Demirbas 2011; Milledge 2011; Benemann 2013; Borowitzka 2013a; Wijffels et al. 2013; Wiley 2013). Challenges associated with industrial-scale production are also being highlighted, particularly for bioproducts with a large market volume but low value (Grobbelaar 2010; Stephens et al. 2013). Three of these challenges – harvesting efficiency, mixing/transportation costs and high water requirements – can be traced back to a fundamental property of microalgal suspension cultures: Low biomass concentration.

In outdoor, production-scale open ponds/raceways, dry biomass concentration (gram dry weight; g(DW)) is typically around 0.5 g(DW) I^{-1} (long term average (Lee 2001), although higher concentrations of up to 50 g(DW) I^{-1} have been achieved in experimental cascade systems (Doucha and Livansky 2009). Enclosed systems typically have higher biomass concentrations; 10 g(DW) I^{-1} have been reached in thin reactor panels under outdoor conditions (Zittelli et al. 2013). The highest reported biomass content for a photosynthetic system was 84 g(DW) I^{-1} using *Chlorococcum littorale* in a 1.6 I laboratory reactor, under constant high-light conditions (2000 µmol photons m⁻² s⁻¹) and pressurised, CO₂-enriched air (Hu et al. 1998). Yet, even at these high biomass concentrations, water is still the main component of the microalgal suspension cultures.

Based on these concentrations, 12-2,000 kg of water are required to produce one kg of dry biomass. Large-scale outdoor production systems are at the high end of this range and processing this quantity of water is a substantial challenge. Water of acceptable quality might not be readily available – especially for freshwater microalgae – or there might be competing demands for water resources from other industries/sectors. Pre-treating marginal water sources or transportation of higher quality water, potentially over long distances, will increase the cost of the production process.

Furthermore a low biomass/water ratio results in high volumes of fluid mixing or pumping during cultivation – for example when moving culture from cultivation vessel to harvesting system. This increases equipment cost and energy use. For most applications, the biomass needs to be dewatered after cultivation, which again increases capital, energy and operating costs. There is considerable on-going research into improving microalgal suspension cultivation, both through increasing the biomass concentration and through improved process design, for example by using more efficient biomass-recovery technologies (Molina Grima et al. 2003). However, great potential lies in investigating a fundamentally different approach by cultivating microalgae as biofilms, i.e. the cells growing colonially attached to a surface at high biomass concentration.

A biofilm is defined as an "Aggregate of microorganisms in which cells are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) adhere to each other and/or to a surface." (Vert et al. 2012). As such, biofilms manifest themselves on surfaces as slimy layers of densely packed cells or as larger flocs on water/air interfaces. Biofilms in natural environments can also include nonmicroscopic organisms, for example macroalgae (Mieszkin et al. 2013), fungi (Blankenship and Mitchell 2006) or higher organisms (Qian et al. 2007). Biofilms can be considered an alternative 'lifestyle' to traditional suspension cultures, as the biomass in the later consists of planktonic single cells or small clusters, chains and filaments. Microalgal biofilms are those dominated by microalgae or cyanobacteria (which we include with microalgae, unless specifically stated otherwise), although other microorganisms can be present in non-axenic cultures – bacteria are almost always present and have been considered essential for microalgal biofilm formation (Barranguet et al. 2005). Microalgal biofilms can be found in a wide range of environments and have been investigated in a variety of fields, e.g. ecology (Thompson et al. 2005), soil biology (Riding 2000), engineering (Schultz et al. 2011) and architecture (Haubner et al. 2006).

The key advantages of microalgal biofilms for biomass production arise from the higher biomass concentration (i.e. high number of cells per unit volume) and the ease with which the attached cells can be separated from their surrounding liquid (Ozkan et al. 2012). High biomass concentration results in reduced cultivation medium requirements, compared to production of the same amount of biomass *via* suspension systems. Consequently, less water is required to be separated from the biomass and to

9

be transported or circulated during the cultivation process. This leads to potential savings in harvesting/dewatering and energy cost. Also, harvesting/dewatering is simpler for biofilms as the cells can simply be scraped (Johnson and Wen 2010) or vacuumed (Craggs et al. 1996b) off the surface, without requiring costly separation techniques like centrifugation or filtration and there is no need to flocculate and settle/float the biomass (Christenson and Sims 2011).

In this article, we provide an introduction into how biofilms can be used to improve microalgal production. We briefly summarise biofilm development, and review biomass productivities of current biofilm cultivation systems. In each section we discuss the implications for biomass production and highlight research gaps and current challenges. We conclude that standardised experimental conditions and species are required for in depth comparison of system biomass productivities and for the identification of drivers that govern biofilm establishment and productivities.

2.3 Biofilm development in a production context

Biofilms – with and without microalgae – are complex structures with features radically different than those of a suspension culture. The change from freely moving cells in a mixed, liquid medium, to a sessile, immobile community adhering to a surface has far reaching effects on the participating organisms, from the beginning of biofilm formation to its maturity.

Microalgal biofilm formation (Costerton et al. 1995; O'Toole et al. 2000) starts when microalgae adhere to a surface. This can either be due to a direct interaction between cells and surface (eg. the adhesion of diatoms) or by secondary colonization of an existing film of macromolecules or bacteria, already adhered to the surface. As the microalgae start growing into microcolonies, they also produce a sticky matrix of EPS. The EPS matrix can also act as a storage compartment for water and other chemicals and can, to some extent, protect the cells against harmful chemicals or environmental conditions (Decho 2000; Flemming and Wingender 2010).

In applied biofilm cultivation, it is critical to understand that the initial adhesion not only depends on the microalgae and the surface material, but also on the cultivation medium and any bacteria that are present in non-axenic cultures. Initial surface

10

interaction has been studied at great length in the context of biofouling (Khatoon et al. 2007; Molino and Wetherbee 2008) and this research can prove insightful, even though its aims are the opposite of those of biomass cultivation.

As the biofilm matures, it grows in height into a three dimensional, multi-layered structure. The cells in these structures are essentially immobilised, and chemical movement of nutrients and waste-products is by diffusion only. Gradients quickly form between the other layers and the depths of the cell clusters and different microenvironments develop around the cells. The cells adapt to these new, local conditions with changes in gene expression and protein activation (O'Toole et al. 2000). For example, in a photosynthetic biofilm, light attenuates quickly, penetrating only a few millimetres from the surface (De Beer et al. 1997). As a consequence, cells at the light-exposed side will adapt to higher light conditions, possible photostress, while those deeper within will adapt to lower light conditions, possibly even heterotrophic conditions. This can have positive effects for productivity, as the adapted cells can make more efficient use of the available light. In contrast, the cells in a well-mixed suspension culture will be constantly moving between zones of different light and will not be able to adapt to a specific light intensity.

In a multi-species biofilm, different organisms can aggregate in zones that are most suitable for them, leading to a heterogeneous structure, often with distinct patterns. An example are microabial mats, where layers of photosynthetic cyanobacteria at the top are followed by heterotrophic and anaerobic bacteria as light and eventually oxygen become limiting (De Beer et al. 1997).

For applied biofilm cultivation, it is essential to be aware of the gradients that exist in biofilms and that measurements in the liquid cultivation medium may not accurately represent the conditions encountered by the cells in deeper layers. Cells in deeper regions of the biofilm can be light or nutrient-limited, which can affect the expression of bioproducts. Depth profiles, for example measuring oxygen status with microelectrodes (De Beer et al. 1997), can provide important information about attenuation rates in the biofilm and the activity of cells in deeper layers. More detailed studies of the structures of biofilms, for example by confocal laser scanning

11

microscopy (Buhmann et al. 2012), are usually limited to small-scale flow cells and are less practical in a production context.

Most biofilms are immersed in a liquid phase, for at least some of the time, and flow is critical for biofilm development. However, the relationship is complicated: On one hand, the liquid needs to move at sufficient velocity to replenish nutrients and carry away waste products, on the other hand, a strong flow will also increase sheer stress on the biofilm structure (Decho 2000). The EPS provides some flexibility, but strong flows can detach single cells, cell clusters or even large parts of a biofilm (aka. sloughing). Turbulent flows usually detach cells from biofilms and limit the maximum thickness of a biofilm to the depth of the laminar boundary layer at the walls of the system (Sakiadis 1961). As cells are carried downstream, they can settle on uncolonized surfaces and grow new biofilms, which can subsequently cause biofouling in undesired locations downstream of the cultivation area. Washed out microalgae are also a dilute, but constant loss of biomass from the harvestable biofilm and will consequently reduce system productivity (Boelee et al. 2011; Zamalloa et al. 2013).

Proper flow management is important for biofilm cultivation. A low flow at the beginning of cultivation can improve cell attachment, while a higher flow may be required at later stages to provide sufficient nutrients for maximal growth (Zippel et al. 2007). It is likely, that microalgal biofilms cope better with gradual increases in flow speed than with sudden increases in sheer stress, although this has not yet been investigated for production systems.

A number of growth models have been formulated, that describe the behaviour of biofilms in production systems (Wolf et al. 2007; Cui and Yuan 2013; Ozkan and Berberoglu 2013; Shen et al. 2013; Murphy and Berberoglu 2014). These models can be used as a foundation for improved process control, scale-up and system design.

2.4 Biofilm-based cultivation systems for microalgal biomass production

Biofilm cultivation systems can be grouped into three broad categories, according to the relative position of the cultivation medium and the microalgae on the cultivation surface. In the first two categories, the microalgae are directly submerged under a layer of medium, either all the time (*constantly submerged systems*, Table 2.1) or some of the time (*intermittently submerged systems*, Table 2.2). The third category (*perfused systems*, Table 2.3) uses a porous substrate that supplies nutrients and moisture to the microalgae which grow on the outside, exposed to the surrounding gas phase.

A comprehensive overview of key characteristics is provided in Tables 2.1 – 2.3, in the following paragraphs we will focus on systems of particular interest. Productivities are reported in grams of dry biomass per square metre of cultivation surface per day (g(DW) m⁻² d⁻¹. For some systems with distinctively non-horizontal geometry, we also report biomass in g(DW) per square metre of foot print per day, defined as the ground area covered by the system when viewed from directly above (plan view), as reported by the respective authors.

Table 2.1: Constantly submerged systems

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Phototrophic biofilm incubator	0.084 (x4)	120*	Modified BG - 11	Natural consortium	~ 2
Zippel et al. 2007	Polycarbonate	16:8	Semi-cont.		~ 14
Phototrophic biofilm incubator	0.084 (x4)	120*	Modified BG - 11	Freshwater consortium	3.63
Guzzon et al. 2008	Polycarbonate	16:8	Semi-cont.	30 °C *	24
Flow-lane incubator	0.0798	90	Modified BG - 11	Cyanobacteria isolates	3.33
Bruno et al. 2012	Polycarbonate	16:8	Semi-cont.	25 °C, 60% RH	25
Biofilm flow cell	0.018	230	Synthetic WW*	WW Consortium	7.7
Boelee et al. 2011	Polyvinyl chloride	24:0	Semi-cont.	25 °C	14
Algae biofilm photobioreactor	0.275	55	BG-11	Botryococcus braunii	0.71
Ozkan et al. 2012	Concrete	24:0	Batch	25 °C	35
Biofilm bioreactor	0.5	88	Municipal WW*	WW Consortium	3.1
Posadas et al. 2013	Foam Polyvinyl chloride	16:8	Batch	24 °C*	30

Table 2.1 continued

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Semi-continuous flat plate	0.009 (1.9)	80	Synthetic W/W/	Nitzschia palea	2.8
photobioreactor	0.008 (x 18)	80	Synthetic www	Scenedesmus obliquus	2.1
Schnurr et al. 2013	N/R	16:8	Semi-cont.	25 °C, 2% CO ₂	20
Attached cultivation system	0.005 (x4)	100	Art. Seawater*	Nannochlorposis occulata	3.87
Shen et al. 2014	Glass fiber reinforced plastic	14:10	Batch	26 °C, 2% CO ₂	14

* = This parameter was varied in the study, we report the best performing combination

N/R Not reported, Semi.-cont.: Semi-continuous, WW: Waste water, BG11: Stanier et al. (1971), Art: Artificial

Table 2.2: Intermittently submerged systems

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Algal turf scrubber	11.49	Outdoor (USA)	Agricultural runoff	Natural consortium	21.6
Adey et al. 1993	Plastic scrubber screen		Continous	Outdoor (USA)	Nov - May
Algal turf scrubber	2.92 (x4)	Outdoor (USA)	Agricultural runoff	Natural consortium	21.6
Adey et al. 1993	Plastic scrubber screen		Continous	Outdoor (USA)	Nov - May
Algal turf scrubber	102		Secondary WW	WW Consortium	60.9
Craggs et al. 1996a, 1996b	Polyethylene	Outdoor (USA)	Continous	Outdoor (USA)	June & July
Laboratory scale algal turf scrubber	1	40-140	Manure WW*	WW Consortium	5.00
Mulbry et al. 2001	Polyethylene	16:8	Semi-cont.	22°C	9 weeks
Benthic algae growth chamber	0.93	40-140	Manure WW*	WW Consortium	5.53
Wilkie et al. 2002	Polyethylene	16:8	Semi-cont.	22°C	9 weeks
Laboratory scale algal turf scrubber	1	300-470	Manure WW*	WW Consortium	Not reported
Pizarro et al. 2002	Polyethylene	23:1 Semi-cont. 23 - 25 °C	23 - 25 °C		

Table 2.2 continued

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Laboratory scale algal turf scrubber	1	240-633*	Manure WW*	WW Consortium	19.10
Kebede-Westhead et al. 2004	Polyethylene	23:1	Semi-cont.	23 - 25 °C	3 - 7 d
Laboratory scale algal turf scrubber	1	40-140	Manure WW	WW Consortium	~7
Mulbry et al. 2005	Polyethylene	16:8	Semi-cont.	23 - 25 °C	42
Laboratory scale algal turf scrubber	1	240-633	Manure WW*	WW Consortium	9.4
Kebede-Westhead et al. 2006	Polyethylene	23:1	Semi-cont.	23 - 25 °C	6 - 8 d
Algal turf scrubber	30		Manure WW*	WW Consortium	25
Mulbry et al. 2008	Landfill liner & nylon netting	Outdoor (USA)	Semi-cont.	< 32 °C	May & June
Rocking attached cultivation system	0.0136	Laboratory	Manure WW	Chlorella sp.	2.57
Johnson & Wen 2010	Polystyrene*	24:0	Batch	20 °C	15
Bench scale rotating algal biofilm reactor	0.244	290	Municipal WW	WW Consortium	1.9
Christenson & Sims 2012	Cotton rope*	14:10	Semi-cont.	19 °C	22

Table 2.2 continued

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]		
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]		
Pilot scale rotating algal biofilm reactor	9.1	Outdoor (USA)	Municipal WW	WW Consortium	14		
Christenson & Sims 2012	Cotton rope		Semi-cont.	Outdoor (USA)	12		
RABR - Enhanced Raceway	5x 0.721	Outdoor (USA)	Municipal WW	WW Consortium	6.57		
Christenson & Sims 2012	Cotton rope	Outdoor (USA)	Semi-cont.	Outdoor (USA)	20		
Photo-rotating biological contractor	1.6	756	Synthetic Acid Mine drainage	Acid mine drainage consortium	0.74		
Orandi et al. 2012	Polyvinyl chloride	12:12	Continous	21 °C	54		
				Scenedesmus dimorphus	0.39		
				Chlorella protothecoides	0.11		
Rocking cultivation chamber	0.019	100	Mod. BBM	Chlorella vulgaris	0.04		
						Scenedesmus dimorphus	0.32
				Scenedesmus obliquus	0.18		
				Chlorococcum sp.	0.53		
Shen et al. 2013	Stainless steel	24:0	Batch	26 °C	15		

Table 2.2 continued

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Rocking cultivation chamber	0.019	100	Mod. BBM	Chlorococcum sp.	4.26
Shen et al. 2013	Glass-reinforced plastic	24:0	Batch	26 °C	11
Algadisk (Rotating biological contractor)	2x 0.045 (x4)	422	M8 -a	Chlorella sorokiniana	20.1
Blanken et al. 2014	Stainless steel mesh*	NR	Batch	38 °C, 15 mM CO ₂ *	7
Pilot-scale rotating algal biofilm system	3.5	642*	BBM	Chlorella vulgaris	4.29
Gross et al. 2013	Cotton duct	Greenhouse (USA)	Semi-cont.	25.5 °C*	May-June*
Laboratory-scale Rotating algal biofilm system	0.045	110-120	BBM	Chlorella vulgaris	3.51
Gross et al. 2013	Cotton duct*	24:0	Semi-cont.	25 °C, 0.03% CO ₂ *	7

* = This parameter was varied in the study, we report the best performing combination.

Semi.-cont.: Semi-continuous, WW: Waste water, BBM: Bold's basal medium; Nichols and Bold (1965), M8 –a: Kliphuis et al., 2010

Table 2.3: Perfused systems

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Twin layer photo-bioreactor	2x 0.67 (x8)	4 - 320 Natural (GER May - September)	Modified f/2	lsocrysis sp. TISO	0.42
				Nannochlorposis sp.	Not measurable
Naumann et al. 2012	Unprinted newspaper		Semi - cont.	Phaeodactylum tricornutum	0.88
				Tetraselmis suecia	0.58
				Greenhouse (GER), < 29 °C	25 (14 for <i>P.</i> tricornutum)
Tube-type PBR	0.09 (12x)	67	Modified f/2	Isocrysis sp. TISO	0.6
				Nannochlorposis sp.	0.8
Naumann et al. 2012	Paper filter	15:9		Ph. tricornutum	1.5
				Tetraselmis suecia	1.8
			Semi - cont.	26.4 °C (22- 34 °C)	25
Twin layer PBR	2x 0.67 (x3)	20 - 220	Municipal WW*	Halochlorella rubescens	1.2
Shi et al. 2014	Nylon filter	Outdoor (GER)	Batch	Outdoor (NL July - September)	8

Table 2.3 continued

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Porous substrate bioreactor	0.0004	110	BG-11	Anabena variabilis	2.8
Murphy et al. 2013	Glass fibre filter	24:0	Batch	25 °C, 0.46% CO ₂	3
Pilot-scale phototrophic biofilm reactor	8.08	Natural (NL Jun - Oct)	Municipal WW	WW Consortium	2.7 - 4.5
Boelee et al. 2013a	Polyethylene woven geotextile		Semi - cont.	Outdoor (NL July - September)	July - September
Vertical phototrophic biofilm reactor	0.125	180	Synthetic WW	WW Consortium	7
Boelee et al. 2013b	Polyethylene woven geotextile	24:0	Semi - cont.	21 °C, CO ₂ in medium	40 (repeated harvesting)*
Filter in Testtube	0.002	10	Modified BBM	Trentepohlia aurea	1
Abe et al. 2003	Chromatography filter	24:0	Batch	25 °C	40
Attached cultivation system	0.06	100	mod. BG 11*	Acutodesmus obliquus	9.16
Ji et al. 2014a	Cellulose acetate/nitrate filter	24:0	Batch	25 °C, 2% CO ₂	7
Attached cultivation bioreactor Type 1	0.08	100	Chu 13	Botryococcus braunii	5.49
Cheng et al. 2013	Cellulose acetate/nitrate filter	24:0	Continuous	25 °C, 1% CO ₂	10

Table 2.3: Continued

System name	Cultivation area [m ²] (Replicates)	Light [µmol m ⁻² s ⁻¹]	Medium	Species	Productivity [g m ⁻² d ⁻¹]
Reference	Surface material	On:Off [h]	Cultivation mode	Conditions [°C, % RH, CO ₂]	Duration [d]
Attached cultivation bioreactor Type 2	1.2	500	Chu 13	Botryococcus braunii	4.91
Cheng et al. 2013	Cellulose acetate/nitrate filter	24:0	Batch	25 °C, 1% CO ₂	10
Attached cultivation bioreactor Type 1	0.08	100	BG-11	Botryococcus braunii	~ 5.6
			BG-11*	Scenedesmus obliquus	9.14
			modified BG-11	Nannochloropsis OZ-1	~ 4.0
			modified f/2	Cylindrotheca fusiformis	~ 4.0
Liu et al. 2013	Cellulose acetate/nitrate filter	24:0	Continuous	30 °C, 2% CO ₂	9
Attached cultivation bioreactor Type 2	0.4	13 - 135 (2)	BG-11	Scenedesmus obliquus	7.1
Liu et al. 2013	Cellulose acetate/nitrate filter	24:0	N/R	30 °C, 2% CO ₂	9
Attached cultivation bioreactor Type 2	1.2	492	BG-11	Scenedesmus obliquus	~ 5
Liu et al. 2013	Cellulose acetate/nitrate filter	Outdoor (PRC)	N/R	2% CO ₂	7

* = This parameter was varied in the study, we report the best performing combination

Semi.-cont.: Semi-continuous, WW: Waste water , BBM: Bold's basal medium; Nichols and Bold (1965), BG-11: Stanier et al. 1971, Chu 13: Largeau et al. 1980, f/2: Walne (1970) N/R: Not reported
Constantly submerged systems are generally constructed as flow cells or channels. In these, the microalgae are grown on a solid surface, covered by a thin layer of medium. Flow is provided by pumping and in most cases, by inclining the flow channel at a small angle. A number of authors (Zippel et al. 2007; Guzzon et al. 2008; Bruno et al. 2012) have used the flow lane incubator proposed by Zippel et al. (2007). This system was designed to provide a standardised laboratory incubator for phototropic biofilms, and included temperature and light sensors for constant monitoring. Guzzon et al. (2008) used this system to investigate the effects of light, temperature and flow velocity on a wastewater consortium, and achieved productivities of up to 3.63 g(DW) m⁻² d⁻¹ with a freshwater consortium, while Bruno et al. (2012) achieved a similar productivity (3.3 g(DW) m⁻² d⁻¹) in one of the few works focusing specifically on cyanobacteria.

The highest biofilm productivity for a constantly submerged system was achieved by Boelee et al. (2011); with up to 7.7 g(DW) m⁻² d⁻¹ in a small flow cell while screening different conditions for wastewater treatment. This system was also used to investigate symbiotic cultivation of microalgae and bacteria under mixotrophic conditions (Boelee et al. 2014).

The system used by Ozkan et al. (2012) is noteworthy for its use of concrete as a cultivation surface, which could be a cheap and easily produced surface material for large-scale in-ground system. However, productivities of *B. braunii* were very low, with 0.71 g(DW) m⁻² d⁻¹. This is possibly due to the low light levels at 55 μ mol m⁻² d⁻¹.

The attached cultivation system described by Shen et al. (2014) is an exception to the usual flow channel designs, as it is essentially the addition of harvestable growth surfaces to a cylindrical, suspension photo-bioreactor . This approach is interesting, as it could enhance existing cylindrical photo-bioreactors, but also it also inherits some of the drawbacks of suspension cultures, such as long optical pathways through medium and a large medium volume relative to the surface area. The geometry also bears some resemblance to the rotating disk reactors (see below), with the disks being fully submerged. Productivity (3.87 g(DW) m⁻² d⁻¹was similar to that of other constantly submerged systems, but the authors achieved much higher productivities (up to 20.53 g(DW) m⁻² d⁻¹) under mixotrophic conditions by feeding glycine as nitrogen and carbon source, in addition to 2% of CO₂.

Intermittently submerged systems come in two varieties: Those based on the Algal Turf Scrubber (ATS, (Adey et al. 1993)) and systems with moving surfaces. ATS and its derivative designs are constructed as flow channels, identical to constantly submerged systems, but the flow rate of the medium is varied to create periodic submersion. This was specifically developed to mimic the wave action experienced by algal turfs on reefs and shorelines (Adey et al. 2011). The advantage of these periodic surges is that the biofilm is periodically replenished by fresh medium, but is directly exposed to light and the gas phase in between waves. ATS systems have shown excellent productivity of 60 g(DW) $m^{-2} d^{-1}$ under outdoor conditions during the summer months in California (USA), although it must be noted that, this biomass had a high ash content (approx 50% average) and that productivity was highly seasonal, and decreased to 4 g(DW) m⁻² d^{-1} during the winter months, resulting in an average productivity 35 g(DW) m⁻² d⁻¹ for the year (Craggs et al. 1996b). The ATS is the most mature and popular individual design for algal biomass cultivation and has been successfully used in wastewater remediation studies, including at very large scale for phosphorus remediation of a creek in Florida (USA) (Adey et al. 2011). The systems economic potential has also been assessed by Pizarro et al. (2006) and (Higgins and Kendall 2012), who found that the cost of wastewater treatment could only be economically viable if the cost is offset by selling algal by-products (Pizarro et al. 2006) or emission trading programs (Higgins and Kendall 2012).

The other variety of intermittently submerged systems encompasses a number of innovative designs in which the surface moves through the (stationary) liquid medium to provide the necessary medium flow for biofilm cultivation. The movement intermittently submerges the biofilm to provide hydration and fresh nutrients and then exposes the cells directly to light and the gas phase the rest of the time. The flow and light dynamics of these systems are quite complex and the constant movement increases operating costs for energy and maintenance.

Johnson and Wen (2010) described the first system of this type, with a rocking cultivation chamber for the production of *Chlorella sp.* from wastewater. This simple laboratory system achieved a productivity of 2.57 g(DW)m⁻² d⁻¹ and was one of the first works to investigate microalgal biofilms specifically for biomass production. The

same approach was also used by Shen et al. (2013), to investigate a range of species and conditions, identifying *Chlorococcum sp.* as the best performing species and increasing its productivity from 0.53 g(DW)m⁻² d⁻¹ to 1.47 g(DW)m⁻² d⁻¹ by optimizing growth conditions. Productivities of up to 4.26 g(DW)m⁻² d⁻¹ are predicted based on a response surface methodology model.

Orandi et al. (2012) adapted rotating biological contactors, which consist of several vertical disks rotating in a trough with medium (Orandi et al. 2012). In acid mine drainage, this system achieved 0.74 g(DW) m⁻² d⁻¹ per cultivation surface, which is equivalent to 8.75 g(DW) m⁻² d⁻¹ per footprint area. A similar design (Blanken et al. 2014), but with only a single disk in each trough has achieved very high productivity of 20.1 g(DW) m-2 d-1 in high light, high CO₂ and high temperature conditions.

The design by Christenson and Sims (2012) uses a rotating wheel encased in rope, with the rope serving as the actual cultivation surface. This system integrates harvesting by continuously running the rope through a harvesting apparatus that scrapes the biofilm off the rope. Scaled up from a benchtop system to pilot scale this system achieved productivities of 14 g(DW) m⁻² d⁻¹ per cultivation surface and 31 g(DW) m⁻² d⁻¹ per footprint area, the ash content of the biomass was, however, not reported. The authors have also tested to enhance existing raceways with rotating biofilm cultivation surfaces and achieved almost half the productivity (6.57 g(DW) m⁻² d⁻¹) while treating wastewater. A later study by Bernstein et al. (2014) investigated photosynthetic parameters of this system in more detail, but did not provide detailed productivity figures.

The rotating algal biofilm system by Gross et al. (2013), is the only system constructed in the manner of a conveyor belt. The cultivation material (cotton) moves around several rotating shafts, passing through a reservoir at the bottom. This system achieved a productivity of 3.51 g(DW) m⁻² d⁻¹, but seems mechanically complex and limited to flexible materials. To our knowledge an earlier conveyor belt system proposed by Cao et al. (2009) has not been implemented.

Perfused systems have also been called 'Twin-layer systems' (Nowack et al. 2005) or recently 'Porous substrate bioreactors' (Murphy and Berberoglu 2014). This approach is characterised by a liquid conducting substratum that supplies nutrients and moisture to the microalgae, which grow on a semi-permeable membrane that acts to stop the microalgae from penetrating into the substratum (Nowack et al. 2005). This allows cultivation with a minimal amount of liquid medium and directly exposes the microalgae to the surrounding airspace, facilitating gas exchange and avoiding light attenuation by medium components between the cells and the light source. However, since the microalgae are not directly submerged, there is an increased risk of drying out for the biofilm and successful cultivation requires a high humidity environment, such as a greenhouse (Naumann et al. 2012).

The twin-layer system was initially developed for biofilm cultivation in 96- well plates (Nowack et al. 2005) and was then adapted to a larger cultivation system (Shi et al. 2007; Naumann et al. 2012; Shi et al. 2014). The system has demonstrated its potential for the cultivation of a number of species, although productivites are lower than those of other systems, with a maximum of 1.8 g(DW) m⁻² d⁻¹ with *Tetraselmis suecia* (Naumann et al. 2012), equal to 10.8 g(DW) m⁻² d⁻¹ per footprint. This might be an inherent limitation of the system, , but could also be explained by less favorable environmental conditions – the experiments were under outdoor conditions in Germany and without additonal CO₂.

A similar approach has been described by Liu et al. (2013) and further used by others ((Cheng et al. 2013; Ji et al. 2014a; Ji et al. 2014b). These systems make use of closely spaced cultivation surfaces to achieve dilution of high light intensities. In a high CO2 environment, this has achieved very high productivites with *Scenedesmus obliquus*, of up to 9.14 g(DW) m⁻² d⁻² and up s of up to 80 g(DW) m⁻² d⁻¹ per foot print.

Murphy and Berberoglu (2014) adapted the twin-layer system into the horizontal porous substrate bioreactor (PSBR), also incorporating their earlier work (Murphy et al. 2013) on microalgal biofilms for life support in space exploration. These experiments were very throughly integrated with a light and mass transport model in order to provide a mathematical describtion of biomass growth and nutrient fluxes. The authors succeded in fitting the model with the experimental data (8 – 16%

average error) and showed the potential for predictive modeling with this approach. The authors have also used the model for the – so far theoretical – design of a vertical production system (Murphy et al. 2014), poetically described as artificial tree.

Boelee et al. (2013a) investigated the largest perfused system to date, a 5x2 m pilot reactor for waste water treatment and achieved productivities of up to 4.5 g(DW) m⁻² d⁻¹, under outdoor conditions. Related work in a smaller system (Boelee et al. 2013b) investigated the effects of harvesting frequency on biomass production, which has received little attention otherwise. Harvesting part of the biofilm every 2 to 7 days resulted in significantly more biomass production (7 g(DW) m⁻² d⁻¹) than one harvest at the end of 20 days (2.7 g(DW) m⁻² d⁻¹).

In summary, at present no clearly identifiable best performing system can be identified, as every design has its advantages and disadvantages and strongly aligned with application. The ATS appears to be generally comparable in suitable applications for traditional suspension culture raceway ponds, while the vertical multi-panel designs for perfused cultivation appear to be comparable with regards to performance and application to vertical PBRs.

2.4 The need for standardisation

Biofilm-based production of microalgae is gaining more attention as a promising alternative to traditional suspension culture, as illustrated by the rapid increase in the number publications over the last few years (see publication dates in Tables 2.1 - 2.3). While the field still retains strong links to its origins in wastewater, there are an increasing number of systems with biomass production as their primary focus and controlled cultivation of a single target species. This has resulted in a number of new and innovative designs that have the potential to fundamentally change our approach to microalgal cultivation.

Unfortunately, there is also a large amount of heterogeneity and lack of standardisation across the field. Most systems are used by a small group of researchers only, within a narrow set of environmental parameters and species – often mutually exclusive to those of other groups. This limits the capability to confidently

make quantitative comparisons between different systems and hinders the identification of the basic drivers of microalgal biomass formation.

Where possible, cultivation systems, at least at laboratory scale, should be tested with a universally used species under common, easily achieved conditions. To obtain general system baseline data, we propose to use the Chlorophyte *Scenedesmus obliquus,* in BG-11 (Stanier et al. 1971), at 120 μ mol m⁻² s⁻¹ of light, 16:8 photoperiod, 25 °C, without CO₂-enrichment. *Scenedesmus obliquus* is a widely used species in microalgal research and has been successfully grown as biofilms in all three types of cultivation systems (Liu et al. 2013; Schnurr et al. 2013; Shen et al. 2013). The proposed conditions should be easily achievable in a laboratory, without the need for specialised equipment, for example provision of CO₂-enrichment. This would facilitate comparison between systems of different design and help to identify beneficial system characteristics.

Reporting of growth metrics for biomass productivity in microalgal biofilms is most commonly expressed as g of g(DW) m⁻² d⁻¹. It is a valuable direct measure where a defined area of biofilm is sampled at different points in time, followed by the biomass being dried and weighed (eg. Johnson and Wen (2010)). Additionally it is recommended to also report ash-free dry weight (AFDW), obtained by combusting the volatile components at high temperature, particularly for biofilms with high proportions of inorganic components (e.g. (Guzzon et al. 2008)). The wet weight of the freshly harvested culture before drying should also be reported, to allow for calculation of water content of the biofilm.

Expressing productivity in g(DW) m⁻² d⁻¹ per footprint is also recommended, as this relates to light use efficiency, which is ultimately limiting photosynthetic production. Productivity per footprint can be calculated for any photosynthetic cultivation system. However, it needs to be noted that reducing a three-dimensional system onto a two dimensional area makes assumptions about the height (or depth) of the system, the direction of the light and the spacing of cultivation surfaces or cultivation vessels, if more than one are present (Slegers et al. 2011). For example, Naumann et al. (2012) calculated productivity per footprint based on 3 vertical 1x1 m panels per m² of footprint, i.e. the average productivity per cultivation surface was multiplied by six. A

system using taller panels or with more closely spaced ones would result in a different multiplier. It is essential that these parameters, assumptions and calculations are clearly communicated.

Rather than dry weight, alternative measurement methods can be required for a specific research context or due to experimental conditions. For example, direct cell counts (Avendano-Herrera and Riquelme 2007), light absorbance (Zippel et al. 2007) or chlorophyll content (Guzzon et al. 2008). These methods can provide benefits in certain applications, such as (i) *in-situ* monitoring, (ii) provide capability to determine biomass in the presence of interfering materials, or (iii) can be of relevance for a specific application. Nonetheless, these measurements should also be presented as dry weights, calculated using conversion factors which have been validated to account for biofilm temporal and spatial variations, e.g. chlorophyll content will attenuate with biofilm depth.

Reporting of physiological characteristics, such as nutrient uptake rates or biochemical profiles is also highly dependent on the research context. Experiments situated in a wastewater context have universally reported nitrate and phosphate uptake. Experiments with single species in controlled condition are likely to omit this information – simply reporting the concentrations in the medium used – but are more likely to investigate the biochemical composition in more detail, especially lipid content and fatty acid profile. We would like to encourage reporting of uptake rates and biochemical profiles, at least in a product potential context, to provide a wider base for comparisons between cultivation systems and with physiological/cell biological microalgal research in general.

2.5 Conclusions

Microalgal biofilms have shown promise to play an important role in the future of industrial photosynthetic biomass production. This review provides a comprehensive overview of the systems in use to date and categorises them into three main types: Constantly submerged systems, intermittently submerged systems and perfused systems. Great creativity in system design was observed, but with this comes a lack of standardisation regarding species and cultivation media used, environmental

conditions and the methods used and metrics reported. Consequently, the result by different groups cannot be reliably compared. We consider the lack of standardisation to be a critical knowledge gap that hinders the development of improved systems for biomass production. Standard species and standardised systems should be used to determine the governing mechanisms in microalgal biofilm establishment, growth and productivities. Standardisation should occur at both laboratory- and pilot-scale. Furthermore, we make recommendation on standardised reporting of biomass baseline productivities, including the necessity to validate biomass proxies for biofilm biomass reporting. Lastly, reporting of nutrient consumption and biochemical profiles will enable comparison with existing microalgal research and techno-economic analyses in new industry contexts.

Chapter 3: A perfused membrane biofilm reactor for microalgae cultivation in tropical conditions

This chapter was published as: "Berner F, Heimann H, Sheehan M (2015) A Perfused Membrane Biofilm Reactor for Microalgae Cultivation in Tropical Conditions; Asian Pacific Confederation of Chemical Engineering (APCChE) & Chemeca 2015, Paper No. 3133047".

The paper was written in its entirety by myself, Kirsten Heimann and Madoc Sheehan provided academic guidance and editorial input.

The findings were presented as an oral presentation at the Asian Pacific Confederation of Chemical Engineering (APCChE) & Chemeca 2015, 27 September – 1 October 2015, Melbourne Australia.

The chapter is presented as published, except for changing the formatting to match that of the rest of this thesis.

Related to this chapter, the rotating perfused membrane biofilm reactor was also subject to a provisional patent application in cooperation with the James Cook University Research office. I provided most of the documentation for this application, in the form of briefs to the research office and in the form of the system description which was adapted into a formal draft application by Denise Hodges, from FisherAdamsKelley. The draft patent application is included as Appendix 3.1

3.1 Abstract

Microalgae have potential to be used as a sustainable raw material in a wide range of applications, such as animal feed, biofuels and green chemistry. New cultivation systems are under development in order to improve productivity and system efficiency. Perfused membrane biofilm reactors are emerging as a potential alternative to traditional suspension systems, addressing the challenges of high dewatering costs. In this approach, the microalgal cells are grown as a biofilm attached to a semipermeable membrane, through which liquid growth medium is supplied. The cells themselves are directly exposed to the surrounding atmosphere. The cultivation surfaces can be arranged as vertical panels, allowing high areal productivity. However, these systems have never been characterised in tropical environments, where high light intensity and extended growth periods offer significant potential for photosynthetic biomass production.

In this work we describe the construction, operation and sampling methodology of a perfused membrane biofilm reactor in Townsville, QLD, Australia (-19.15°S, tropical Savannah climate). A biofilm of *Mesotaneium sp.* was successfully cultivated under greenhouse conditions and growth curves were obtained. Maximal biomass productivity of up to 1.7 g.m⁻² d⁻¹ (dry weight) and a maximal biomass yield of 21.25 g.m⁻² (dry weight) were recorded. Testing procedures were used to assess spatial variations in growth. Considerable differences between samples from different locations on the vertical panels indicate the need for structural design modifications to the reactor. Results are put in context to existing research and adaptations to deal with high temperatures and high evaporation rates in tropical regions are discussed.

3.2 Introduction

Microalgae are gaining increasing attention as a bio-resource for photosynthetic production in a wide range of applications. This includes large-scale bulk products, such as biofuels; highly refined products in pharmaceutical and green chemistry; and products for humans and animal consumption, such a nutraceuticals, agriculture and aquaculture feeds. In addition, this production can be tied in with the recycling of carbon dioxide (CO₂) and remediation of waste waters, enabling synergistic and sustainable production pathways across a variety of industries (e.g. (Milledge 2011; Benemann 2013; Borowitzka 2013a)).

A critical challenge to improved productivity and system efficiency is the low culture concentration of traditional pond or tank cultivation systems (Molina Grima et al. 2003). The usable biomass content in these systems is often far below 1% of total culture weight. This large proportion of 'unproductive' weight (i.e. water) results in higher energy cost for mixing and pumping during cultivation and leads to substantial costs for concentrating and dewatering in biomass production processes.

Biofilm cultivation systems employ an alternative approach to cultivation by growing the microalgae attached to a solid support or membrane, instead of freely suspended in liquid medium. The key advantage of biofilms lies in the higher cell concentration compared to suspension cultures, which greatly reduces harvesting effort as the cells can simply be scraped (Johnson and Wen 2010) or vacuumed (Craggs et al. 1996b) off the surface and can be used without the need for further concentration (Ozkan et al. 2012). Furthermore, the higher cell concentration reduces the amount of liquid medium needed for a given amount of biomass, leading to both lower water requirements and reduced energy cost for preparation, mixing and removal of the medium.

Perfused membrane biofilm reactors (PMBR) are among the numerous biofilm cultivation systems that have been described in the last years - see Berner et al. (2014)¹ for an general review. Also known as twin-layer systems (Naumann et al. 2012), PMBR (Figure 3.1) consist of a liquid conducting material – currently glass-fibre

¹ Chapter 2 of this thesis

capillary matting – which supports a liquid permeable membrane. The microalgal cells are inoculated onto this membrane and draw liquid medium and the necessary nutrients through the membrane. Key advantage of this arrangement is the separation of nutrient medium and cells, which allows medium recirculation without cross contamination (Naumann et al. 2012). In addition, the biofilm is directly exposed to the surrounding atmosphere, without an intervening layer of cultivation medium. This avoids light attenuation due to medium components and allows for rapid exchange of CO_2 and oxygen (O_2) between cells and atmosphere (Murphy et al. 2014). The main disadvantages are the higher mechanical complexity of these systems, the cost of the membranes and the need for suitable environmental conditions – especially humidity – to avoid drying out of the biomass.





The microalgal biofilm (green) grows on a semi-permeable membrane (horizontal hatching) on top of a liquid conducting base layer (blue crosshatching). The microalgae are directly exposed to light and CO₂. The membrane allows the passage of water and nutrients, but prevents the cells from passing into the base layer, illustrated by the dark green ellipses and the red crossed arrows.

In contrast to other biofilm reactors, the cultivation surfaces can be arranged as vertical panels, which allows for a large cultivation area per land footprint and consequently high areal productivity. In addition, the panels can be spaced to 'dilute' the available light over a given amount of surface area to avoiding photo-damage and

growth inhibition in high light intensities (Liu et al. 2013) which is especially important in lower latitudes where light intensity is very high.

The flexibility to mitigate high light concentrations and the need for high humidity would make PMBRs particularly suited for tropical latitudes where high light intensities can be found alongside high temperature and humidity. However, all current research on PMBR under outdoor conditions has been conducted in temperate conditions in Germany (Naumann et al. 2012; Shi et al. 2014; Schultze et al. 2015), the Netherlands (Boelee et al. 2011) and China (Liu et al. 2013). To address this deficiency, we describe the design, construction and operation of a PMBR in Townsville (Australia) within a tropical savannah climate.

3.2 Design and construction of the perfused membrane biofilm reactor

The design of the perfused membrane biofilm reactor (PMBR) was adapted from Naumann et al. (2012) with the microalgal cells growing on a membrane on top of a liquid conducting capillary matting (Figure 3.2). The capillary matting provided nutrients (Isola AS, Eidanger, Norway, matting weight 80 g m⁻²) and water supply while the membrane stopped the cells from penetrating into the capillary matting. The capillary matting was mounted in PVC frames (manufactured locally) for added stability. Both single panels (Figure 3.2) and a novel rotating system with 4 panels were constructed (Figure 3.3). Culture medium, provided in a trough on top of the system, flows through the matting by capillary action and gravity. Medium was collected at the bottom and recirculated to the top by a peristaltic pump, controlled by a float switch in the top trough.



Figure 3.2: Details of the vertical cultivation panel

Top left: The cultivation panel mounted in a greenhouse, with freshly inoculated filters with *Mesotaenium* sp. (NQAIF 303).

Bottom left: Flow of the medium in the vertical panel. Medium supplies the capillary matting from the top and flows downwards. Medium is constantly recirculated by a peristaltic pump.

Right: Technical drawing of the panel, including dimensions in mm.

47 mm glass fibre filters (GF/A MicroScience MS GA) were used as membrane material. The filters were placed on both sides of the wetted capillary matting and would stick without the need for further attachment, as long as the matting remained moist. A key advantage to the use of filters is that these filters could be inoculated with a precise and reproducible amount of biomass by vacuum filtration and the whole (tared) filter could be removed and dried to determine biomass dry weight, without need for scraping and potential contamination of the biomass by membrane material.

For single panel cultivation, the panel was mounted vertically, in approximately north/south direction in a greenhouse in Townsville Qld, Australia. The greenhouse was equipped with limited climate and humidity control, by means of an inbuilt air-conditioning system and an added atomising humidifier (AirEven Condair 505S) (Figure 3.4 for climate profiles). No artificial light was used, resulting in a light period of approximately 12 h light to 12 h darkness and average light intensity of 302 μ mol photons m⁻² s⁻¹ and maximum intensities up to 4887 μ mol photons m⁻² s⁻¹.

The rotating system (Figure 3.3) consisted of four vertical panels mounted on central shaft, with a shared medium supply and collection above and below, respectively. In order to provide intermittent illumination to all panels, a geared electric motor (Maxon RE40) was installed to rotate the system at 3 rpm. With four panels, this mode of operation resulted in full illumination for ¼ of each rotation (s) and shade for the remaining period.





Top left: 3D rendering of the system that served as the basis for the construction. *Top right:* The rotating frame of the system, without the panels installed. The motor for the rotation and the pump for medium recirculation are housed in the socket at the bottom.

Bottom left: Assembled system with panels installed for the first time. The wrinkles were avoided in later assemblies. A light logger (blue) is tied to the central shaft. *Bottom right:* Close up of the beginning of a later experiment, with the microalgae airbrushed onto the smooth paper surface.

3.3 Material and Methods

For the single panel experiment, the panel was installed with the capillary matting in place and disinfected by recirculating a mix of 70% ethanol and 30% deionised water for 16 hours (overnight). The system was then emptied, and left to air dry for 4 hours, before being primed by recirculating Bold's basal medium (BBM, (Nichols and Bold 1965)), for one hour to ensure that the capillary matting was thoroughly soaked.

A starter culture was prepared by cultivating a locally isolated strain of *Mesotaenium* sp. (NQAIF 303) in BBM in an aerated 2 l bottle for 7 days. 7.5 ml of this starter culture were vacuum filtered onto pre-ashed and tared 47 mm GF/A filters, for an initial biomass of 0.0016 g ash-free dry weight (AFDW, see Eq. 3.1) per filter, equivalent to 1.16 g AFDW m⁻². 36 filters were placed on each side of the panel.

The biomass was cultivated for a total of 26 days, with medium being replaced every 7 days. In order to test for differences along the length of the panels, each panel was divided vertically into six zones, each zone containing six filter papers. Filters were sampled by removing one filter from each of the six zones per side, with filters chosen randomly within each vertical zone. Removed filters were replaced with fresh filters, inoculated as above, in order to preserve some biomass load – these replacement filter were not sampled.

In order to enable dry biomass determination, the filters were pre-ashed (500°C, overnight) and tare weight determined before the cultivation (TW). After sampling, the filter was immediately weighted to determine wet weight (WW) and then dried at 105°C in a microwave asher (Milestone Pyro) for 4h. After 1 h of cooling in a desiccator, the filter was weighted again to determine dry weight (DW). The organic components were burnt off at 500°C overnight, allowing the ash weight (AW) to be determined.

Ash free dry weight (AFDW) [g filter⁻¹]was calculated as follows:

$$AFDW = DW - AW \tag{Eq. 3.1}$$

AFDW per filter was converted into g m⁻² (AFDW) based on the inoculated area of each filter (0.0014 m²). Specific growth rates (SGR, $[d^{-1}]$) were calculated by fitting a linear regression to the appropriate sections in a semi-logarithmic plot of AFDW versus time in days.

Total solid content [%] (TSC) was calculated as follows:

$$TSC = \frac{(WW - TW) - (AFDW - TW)}{(WW - TW)}$$
(Eq. 3.2)

Temperature and humidity next to the cultivation panel were recorded (Thermochron HC). Light levels were measured at the beginning and the end of the cultivation period (LICOR LI-250A).

For the rotating systems, four panels were installed and prepared as described above. Unprinted newspaper was used as cultivation surface. 45 ml of mother culture were concentrated by centrifugation (Eppendorf 5810) to 10 ml. A commercially available, gravity-fed airbrush was used to spray this concentrate onto each panel, allowing for even coverage and minimum overspray with some care and practice. While this method was considered less precise than vacuum filtration, it still allowed a defined amount of biomass to be applied onto the surface.



Figure 3.4: Temperature and relative humidity during vertical panel cultivation of *Mesotaenium* sp. biofilms.

Despite the constant operation of a humidifier during the spring season in Townsville, Queensland, Australia, it was not possible to avoid decreases in humidity when temperatures rose above 35°C during the day.

3.4 Results

The growth trials showed that perfused cultivation of microalgal biofilms under tropical greenhouse conditions was possible. However, the experiments also indicated a number of complications, due to the high temperatures experienced (above 39 °C).

In the single panel system, average biofilm growth followed a similar pattern on both surfaces (Table 3.1, Figure 3.5), showing 3 distinct growth phases – a first exponential growth phase for the first four days, followed by a second exponential growth phase until day 14, and a pronounced stationary phase until day 26 (diauxic growth). Maximum biomass production was higher on the western side of the panel with of 21.25 g m⁻² (AFDW) at day 14 compared to 19.33 g m⁻² (AFDW) on day 21 on the eastern side (on day 14, the eastern side had 17.55 g m⁻² (AFDW)). Maximal

productivity was 1.70 g m⁻² d⁻¹ (west) and 1.36 g m⁻² d⁻¹ (east), respectively. Average TSC of the biofilms increased from 1.6 % at the beginning of cultivation to 6.3% on day 14.

Ū		•			•			
		Day 0	Day 4	Day 7	Day 11	Day 14	Day 21	Day 26
		Avg. ADFW	ADFW					
_		[g m ⁻²]						
East side	Average	1.16	3.97	6.55	11.72	17.55	19.33	14.25
	Std. Dev.	0.00	0.19	0.42	1.46	1.85	5.56	7.99
West side	Average	1.16	4.26	7.92	16.29	21.25	20.63	22.58
	Std. Dev.	0.00	0.33	0.76	1.24	2.13	7.82	7.68

Table 3.1 Average biomass production in a PMBR

Average biomass production across the whole panel and standard deviation.





Specific growth rates (SGR) under greenhouse conditions are shown for each growth phase. Both sides of the panel follow an almost identical growth pattern with rapid increase in biomass in the first 4 days, followed by a slower growth phase until day 14 and a stationary phase until day 26.

During cultivation it became apparent that there was considerable difference in growth between individual filters (Figures 3.6 & 3.7) and that vertical location affected growth performance. In some cases, there was evidence of biomass sloughing off the filter (Figure 3.6, white stars), leading to loss of biomass on specific filters and contamination of matting and lower filters.



Figure 3.6: Filters on the east side of the panel of the PMBR, with *Mesotaenium* sp. biofilms on day 21

The dashed lines and white numbers indicate the different zones. The stars mark filters with substantial sloughing.

Left: Overview of the whole panel. The dark green filters had been cultivated for 21 days, the lighter green ones are replacements of harvested (removed) filters.

Right: Detail of sloughed and dried out biofilms in zones 5 and 6. The lowest four filters are inoculated spares that were cultivated for pre-trials of methods, but not used in the experiment.



Figure 3.7: Deviation of biomass of *Mesotaenium* sp. from average for the six different zones of the vertical PMBR

Shown is the difference in AFDW for filters from each vertical zone along the panel, relative to the average of all filters sampled on that day. The filers in the lower zones (especially 5 & 6) are consistently below average weight. Overall deviation increased over the course of the experiment.

Furthermore there was systematic bias in growth along the vertical length of the panel (Figures 3.6 & 3.7). Biofilms at the top of the panel stayed hydrated throughout the cultivation period while those at the bottom of the panel showed signs of drying out after only a few days and were completely dried out after the second week of

cultivation. This lead to a systematic difference in growth. As such, the standard deviations presented in Table 3.1 are to be used with caution, as these calculations assume unbiased distribution around the average (Van Emden 2012)

The cause of this can be traced to insufficient liquid supply due to high evaporation from the panels, associated with the rise in temperatures during the day and the corresponding drop in relative humidity (RH), often below 50% RH (Figure 3.4). These fluctuations could not be compensated by the greenhouse's air-conditioning system, despite the use of the atomising humidifier.

The trial of the rotating system was even more severely affected by the environmental conditions, as it took place closer to the peak of the southern hemisphere summer. Temperatures in the greenhouse rose above 50 °C during this experiment and all panels dried out within the first three days, before the first sampling date. However, visible growth during this initial period showed that *Mesotaenium sp.* could be successfully applied by air-brushing, with the cells remaining viable despite the harsher conditions. Due to the need for improved liquid conduction in the prototype and overall environmental control, the experiment has not been repeated to date.

3.5 Discussion

The experimental work has provided useful insights into the potential and complications associated with perfused membrane biofilm cultivation in tropical climates.

Areal productivity (expressed here as g (AFDW) per m² of ground per day) would depend on the arrangement and number of cultivation panels. Assuming that the current growth rates can be sustained, a system of 3 double-sided, 1 m tall and 1 m wide panels at a distance of 0.3 m - as demonstrated by Naumann et al. (2012) - would provide 6 m² of cultivation surfaces per m² of land and a correspondingly increased total biomass yield. Based on the 14 day-biomass data presented, such a system would have a productivity of 7.5 to 9.1 g m⁻² d⁻¹ (AFDW). Since irradiation was high, even closer spaced panels could be employed, making use of light dilution to avoid photo-inhibition (Liu et al. 2013). Improvement in liquid conduction would allow for taller panels with the higher growth rates currently found at the top of the panels

being retained across the entire length. Based on these assumptions, a case scenario of 10 panels per m² of ground, each 2 m tall – i.e. 40 m² of production surface - could result in productivities up to 66.8 g m⁻² d⁻¹. However, considerable system engineering and process improvement would be needed to make such a system workable.

In other PMBR systems, productivities of up to 52 g m⁻² d⁻¹ (AFDW) have been experimentally achieved under outdoor conditions in Germany (Schultze et al. 2015) and up to 88 g m⁻² d⁻¹ (AFDW) under indoor laboratory conditions (Liu et al. 2013), although it needs to be noted that these experiments (and the ones presented here) are only common in terms of the general cultivation method. Growth comparisons would depend on similarity with respect to species, light conditions, nutrients and environmental conditions. Furthermore, direct comparison is complicated by the complex relationship between system geometry and light availability.

The biomass concentrations in commercial, large-scale open suspension systems, are typically around 0.5 g Γ^1 (long term average, (Lee 2001)) – equivalent to a TSC of 0.05%. Higher concentrations have been achieved in experimental systems with up to 50 g Γ^1 , 0.5% TSC (Doucha and Livansky 2009). For primary harvesting, these cultures would be concentrated (by centrifugation or filtration) to a TSC of 2 to 7% (Uduman et al. 2010); comparable to the 6.3% TSC that was achieved using the presented cultivation method without any processing. This represents a 263-fold increase in biomass density, compared to the concentration of the suspension culture used to inoculate the system. Consequently, PMBRs can lead to economic benefits by eliminating primary dewatering steps.

The failure of the rotating system was due to environmental conditions and not due to mechanical unsuitability. Intermittent illumination has shown to increase productivity and biomass quality in suspended cultures (Grobbelaar 1989; Xue et al. 2011; Yoshioka et al. 2012), but has not yet been studied in a biofilm production context. As such the concept of radially arranged, rotating cultivation surfaces that allow for intermittent illumination remains of interest. However, it needs to be noted that the frequency of intermittent illumination is critical and that enhancement in suspension cultures occurs at frequencies of greater than 1 Hz (Grobbelaar 1989; Xue et al. 2011), although Xue et al. (2011) has shown some effect at frequencies between 0.1 and 1 Hz. For a

rotating biofilm system, this would require the system to rotate at 6 to 60 rpm (for 0.1 to 1 Hz, respectively) or even faster. This is 2 to 20 times faster than possible with the prototype used in this work and would pose an additional challenge regarding the stability of the system and the effect of the resulting centrifugal force on the biofilm and the liquid medium. Consequently, the development of such a system would require an interdisciplinary approach between mechanical engineering, fluid engineering and biological science.

Temperature and humidity play an important role during cultivation, but can only be controlled if the cultivation takes place in a greenhouse with appropriate environmental control. Under tropical conditions a degree of shading would be required in order to allow for efficient temperature control. By necessity, this will also mean a reduction of light level. At very high light intensities, this might even be beneficial for biomass production, as it avoids or reduces light inhibition of the microalgal cells. If the biomass and system design have been optimised for high light cultivation – by cultivating high light tolerant strains under appropriate light dilution – a reduction in light intensity would be lead to a loss of productivity.

Cultivation in a greenhouse would also require humidity control which would add to the total water use of the system. Alternatively, an increase in the rates of liquid conduction could be used to compensate for the high rates of evaporation. The addition of misters or sprinklers to keep the biofilm moist in periods of low humidity, is also a possible design feature. However, this might not be necessary under more humid tropical climates at lower latitudes, such as 'wet tropics', rainforest or monsoon climate. Omitting a greenhouse would reduce construction and maintenance cost, although at increased risk of airborne contamination and generally increased exposure to harsh environmental factors.

3.6 Conclusions and recommendations

Perfused membrane biofilm reactors offer interesting possibilities for microalgae cultivation in tropical climates, promising advantages regarding water consumption and harvesting effort. However, further research is needed to increase the fundamental knowledge of these systems and in order to develop sustainable

applications. The understanding and control of the internal liquid conduction in the capillary matting needs to be improved, to provide optimal growth conditions for the microalgae with minimal effort for environmental control. In-depth understanding of light and shading for different systems can lead to innovative designs that make efficient use of the abundant light for maximal productivity.

Microalgae are renowned for their cosmopolitan distribution and environmental hardiness, and local strains that are tolerant and productive under local conditions should be isolated, characterised and assessed for industrial production. However, the use of common strains should also not be neglected, in order to provide meaningful comparisons to work done in other parts of the world. As tropical regions are developing economically around the world, perfused membrane biofilm systems can help by providing an efficient and sustainable way to produce a versatile bioresource.

3.7 Acknowledgements

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Chapter 4: Petri-dish assays as a research tool for perfused microalgal biofilms

4.1 Abstract

A Petri-dish assay (PDA) was developed to provide a standardized cultivation and testing method for perfused microalgal biofilms. The use of this assay was successfully demonstrated in several experiments, showing the difference of growth behavior of different species of microalgae in biofilm and suspension cultures, as well as the interaction between growth and materials in different species and the effect of outdoor vs. indoor conditions. The assay was also used to examine the time dependency of conversion factors between cell numbers and dry weight. To conclude, the PDA presents a simple and relatively inexpensive tool for microalgal research. There is potential for wider uptake across the field of microalgal biofilm research, in order to achieve a higher degree of standardisation between different research groups.

4.2 Introduction

Microalgal biofilm cultivation is emerging as a promising alternative to traditional suspension systems. In biofilm-based cultivation, the microalgal cells are grown attached to a surface at high cell density. This results in reduced water requirements and increased harvesting/dewatering efficiency, addressing two of the key challenges of microalgal cultivation. As such, biofilm cultivation has the potential to lead to reduced production cost and economic benefits across a number of applications, such as biofuels, agriculture and aquaculture feeds, and specialty biomolecules (Christenson and Sims 2011; Milledge 2011).

A number of innovative biofilm cultivation systems have been described in recent years (for reviews, see Berner et al. (2014) and Gross et al. (2015a)), proposing a wide range of geometric arrangements, environmental and nutrient conditions. These systems can be categorized based on the movement of the biofilm cultivation surface (stationary or mobile, (Gross et al. 2015a)) or based on the flow of the liquid medium relative to the biofilm surface (Berner et al. 2014), with systems being divided into constantly submerged systems, intermittently submerged systems and perfused systems.

In perfused systems, the biofilms are grown on a semi-permeable membrane, which separates the cells from the bulk medium supply. In this way, the cells are directly exposed to the surrounding air phase, without an intervening liquid layer. This reduces attenuation of light and eliminates limitations associated with the low solubility of CO₂ (Sydney et al. 2010) in the liquid medium, avoiding carbon limitation at high growth rates. In addition, the separation of the liquid conducting material from the cells avoids unwanted cell growth in the liquid phase and allows for greater control of water usage (Naumann et al. 2012).

Perfused biofilm systems were originally intended as a very small-scale bio-sensor system (Podola and Melkonian 2003) which was then developed into a 96-well platebased cultivation system (Nowack et al. 2005) for culture collections. Successively larger systems were later developed for mono-species production (Shi et al. 2007; Naumann et al. 2012; Schultze et al. 2015), achieving very high growth rates of up to

31 g m⁻² d⁻¹ (based on cultivation surface area) by incorporating high light and high CO₂ enrichment. A parallel development emerged in China, where several experiments (Liu et al. 2013; Cheng et al. 2014; Zhang et al. 2014) achieved high growth rates (up to 9.1 g m⁻² d⁻¹), by incorporating several closely spaced cultivation surfaces, to make use of light dilution in high light conditions. Murphy et al. (2014) investigated cyanobateria cultivation and established a theoretical description of perfused biofilm cultivation (Murphy and Berberoglu 2014). An outdoor pilot-scale system for wastewater treatment (Boelee et al. 2013a; Boelee et al. 2013b) showed the basic potential for mixed culture cultivation, although nutrient removal was ultimately not considered to be satisfactory.

Although research and understanding has progressed, it needs to be noted that each system (or system family) has been designed in isolation, within its own specific area of interest and there is little overlap with regards to experimental conditions. Unfortunately, this leads to results not being directly comparable between different systems, due to the number of compounding factors affecting the overall outcomes. For example, even though the experiments by Naumann et al. (2012) in Germany and Liu et al. (2013) in China used similar systems for perfused cultivation of mono-clonal microalgae on filter membranes, it is impossible to directly identify the driving factors behind the better growth performance of the latter (Table 4.1). The cultivation conditions differed in several aspects and it cannot be determined with certainty which of the factors were responsible for or contributed to the observed differences. This limits the ability to deduct how these systems would behave in other circumstances. For example, how would *Isochrysis sp.* (used by Naumann et al. (2012)) grow in the multiplate system (Liu et al. 2013)? Is the addition of CO₂ critical for the faster growth of *Nannochloropsis*?

This lack of standardisation is not limited to these specific systems, but rather applies to the whole field of applied biofilm research. The great creativity in system development has been accompanied by a distinct lack of comparability between different approaches. Consequently, as the divergence between designs increases, it becomes increasingly difficult to transfer knowledge under one set of circumstances to another. Even basic questions about the specific effects of cultivation conditions or

system components (e.g. the effects of different surface materials) cannot be reliably answered based on the current data, hampering the identification of fundamental drivers of microalgal biofilm cultivation. Standardisation of pilot- or full-scale cultivation systems would be expensive, time consuming, and would limit creativity in design. Detailed plans for existing systems are often hard to come by and while the major dimensions are well published, minor details, such as specific grades of the material used are harder to find and can be subject to confidentiality due to current or planned intellectual property protection.

Table 4.1: Com	parison of two	different cultivation s	ystems.
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System	Tube-type PBR	Single layer vertical plate attached photobioreactor		
Published in	(Naumann <i>et al.</i> 2012)	(Liu <i>et al.</i> 2013)		
	Phaeodactylum tricornutum	Scenedesmus obliquus (local		
	UTEX 642 (marine)	isolate, FW)		
	Tetraselmis suecica strain			
Spacias	PLY 305 (marine)	Botryococcus braunii SAG (FW)		
species	Isochrysis sp. T.ISO (CCAP	Cylindrotheca fusiformis		
	927/14) (marine)	(Chinese isolate, FW)		
	Nannochloropsis sp. (CCAP			
	211/78) (marine)	Nanochloropsis OZ-1 (marine)		
Cultivation		Freshwater: BG-11 (Stanier et al.		
medium	f/2 (per Walne (1970))	1971) Marine: f/2 (Walne 1970)		
System	12 0.9 x 0.1 m modules with	0.4 x 0.2 m plate with		
dimensions	18 filters each	unspecified number of filters		
		Cellulose acetate/nitrate		
Surface material	Paper filter	membrane filter		
Light (on:off)	67 μmol m ⁻² s ⁻¹ (15h:9h)	100 μmol m ⁻² s ⁻¹ (24h:0h)		
Light source	Sodium discharge lamps	Fluorescent lamps		
Gas	Ambient air	Enriched with 2% CO ₂		
Temperature	26.4 °C	25 °C		
Inoculation	2	2		
density	2 g m ⁻²	10 g m ⁻²		
	<i>P. tricornutum:</i> approx.~ 38	2		
Biomass growth	g m ⁻² in 20 d)	<i>S. obliquus:</i> 73.1 g m ⁻² in 8 d		
during	<i>T. suecica</i> : \sim 31 g m ⁻² in 14 d	<i>B. braunii:</i> ~ 48 g m ⁻² in 8 d		
exponential phase	Isochrysis sp. TISO: ~14 g m ⁻²	2		
Max Growth rate	in 16 d	C. fusiformis: ~ 30 g m ⁻² in 7 d		
$(g m^{-2} d^{-1})$	Nannochloropsis sp.: ~ 5 g m			
	⁻ in 10 d	<i>Nanochloropsis</i> : ~30 g m ² in 7 d		
	P. tricornutum: 1.8	S. obliquus: 9.1		
	T. suecica: 1.5	B. braunii: 5.7		
IVIAX Growth rate	Isochrysis sp. T.ISO: 0.6	C. fusiformis: ~ 4.2		
(g m ⁻ a ⁻)	Nannochloropsis sp.: 0.8	Nanochloropsis: ~4.2		

All weights are presented in dry weight (DW).

In view of these limitations, we suggest that there is value in designing simpler benchscale approaches, which allow for the isolation of specific factors relevant to microalgal growth and can be used to generate initial comparative data in standardisable conditions which can then be used to justify more substantial investments into advanced cultivation systems.

Key development criteria for such assays are:

- The method is easily established and replicated in phyoclogical laboratories.
- Use of material and procedures familiar to biologists or easily learned.
- Use of standardized, off-the-shelf materials, without the need for bespoke manufacturing.
- Use of existing laboratory infrastructure and equipment to reduce initial investments
- Allow for independent replications
- Provide sufficient biomass for quantitative and qualitative analyses by standard methods, such as generation of biomass growth curves, or determination of lipid, FAME and protein contents.

In this work, we developed a test method for cultivating perfused microalgal biofilms in Petri-dishes. Petri-dishes are well established in microbiological laboratories, are inexpensively obtained and are familiar to most biologists. The experimental design is based on similar tests that have used filters on agar for the cultivation of bacterial biofilms (Merritt et al. 2005) and for *in-situ* cultivation of biofilms in environmental studies in streams and rivers, e.g.(Marcarelli and Wurtsbaugh 2006; Scott et al. 2009), but not specifically for the cultivation of mono-species microalgal biofilms. While mono-species cultivation microalgae on agar is well established as a technique in culture collections (Lorenz et al. 2005), this use doesn't involve filters and is more aimed at maintaining the microalgae than rapid growth.

The Petri-dish assay was used to investigate and comparisons of basic cultivation parameters (species, cultivation conditions, surface material) and as a method to determine the potential to provide conversion factors for use in different fields of application.

The experiments were designed to showcase how the Petri-dish assay can be used to investigate different aspects of perfused microalgal cultivation. In each test scenario, Petri-dishes were set up to allow for triplicate measurements at several time points during a 3 to 4 week trial. Four different aspects were investigated: Growth of different species of microalgae in biofilms and suspensions culture, cultivation indoors versus tropical outdoor conditions, growth on different materials and establishing growth curves to calculate time-sensitive conversion factors between different analytical methods. The specific details for each scenario are outlined below.

4.3 Test scenarios

The overall concept of the Petri-dish assay (PDA) was to inoculate a defined quantity of microalgal cells onto a semi-permeable material (paper, later membrane filters) which was placed on top of an agar plate to supply the necessary nutrients by diffusion (Figure 4.1).



Figure 4.1: Overview of the Petri-dish assay The microalgal biofilm grows on top of a semi-permeable membrane, while being supplied with nutrients from the agar plate. Light was provided from the top (sun symbol).

The PDA was used in four basic

scenarios that showcase its use in answering fundamental questions typically occurring in the early stages of biofilm application research.

Scenario 1: Growth behavior of different species and comparison with suspension cultures

Four locally isolated species of microalgae (*Mesotaenium sp., Scenedesmus quadricauda, Tetraedron sp., Desmodesmus armatus*) were grown as biofilms and suspension cultures. The capability of a species to grow as a PDA biofilm and growth performance in comparison to the well-established suspension systems is a common and fundamental question in the early stages of a biofilm project. Direct comparisons of growth under identical environmental conditions – ideally next to each other – provide a straightforward answer.

It is necessary to note that different metrics are used to measure biomass in suspension systems and biofilm systems. In suspension cultivation, biomass concentration is expressed per volume (cells Γ^1 and g Γ^1), while in biofilm cultivation biomass concentration is expressed per area (cells m^{-2} and g m^{-2}). While the conversion of between volume and area is mathematically straightforward if the system dimensions are known, it needs to be noted that the result is highly dependent of the height (or depth) of the culture and that unrealistic cultivation expectations can easily lead to bias and should be used with care. For this reason it is generally preferable to use relative values where the increase in biomass is calculated relative to the initial biomass present in the system. Where direct comparisons between biofilms and suspension cultures are made in this work, the relative biomass growth (RBG) is expressed as ratio of the current biomass concentration compared to the inoculum biomass concentration (Eq. 4.1):

$$RBG_t = \frac{X_t}{X_0} \tag{Eq. 4.1}$$

With X_0 (cells m⁻² or cells l⁻¹) being the cell concentration at the start of the experiment and X_t being the cell concentration on a given day.

Also used for comparisons is the specific growth rate (SGR or μ ; [d⁻¹] (Becker 1994)).

$$SGR = \mu = \frac{\ln(\frac{X_1}{X_2})}{t_2 - t_1}$$
 (Eq. 4.2)

With X_1 and X_2 being the biomass concentration in the system on day t_1 and t_2 , respectively. This formula can be applied regardless of the metric used for determining biomass concentration.

Scenario 2: Indoor vs Outdoor conditions

Mesotaenium sp. was grown in a greenhouse under natural sunlight and compared to growth under laboratory conditions. An indication of growth performance under outdoor conditions is essential in deciding if further research and investment into larger outdoor cultivation systems is advisable. Small-scale trials can provide basic insight into the duration of the growth phases and can indicate potential complications that would not occur in a laboratory set-up, such as contamination or exposure to inhibitory light levels and temperatures. These factors can then be addressed during the design of the outdoor prototype.

Scenario 3: Biofilm growth on different materials

The growth of three different species (*Mesotaenium sp., Isochrysis* aff. *galbana* (TISO), *Prymnesium* sp.) was compared on two different membrane filter materials, fiberglass (FG) and polyvinylidene fluoride (PVDF). In order to allow for direct comparisons, the difference in DW [g m⁻²] was calculated (Eq. 3)

$$\Delta DW = DW_{PVDF} - DW_{FG} \tag{Eq. 4.3}$$

Positive values in this equation indicate higher biomass on PVDF, negative values higher biomass on FG.

The choice of surface material is import for biofilm cultivation, as chosen surfaces must be suitable for biofilm growth and should also satisfy process-specific requirements, such as harvestability or compatibility with analytical methods (eg. temperature tolerance for dry weight). Economic factors, such as durability and cost also need to be considered. However, most research on the interaction of material and microalgal biofilms has been conducted in the context of preventing biofouling and has consequently aimed to minimize growth of mixed biofilms on structural materials in uncontrolled conditions – almost a direct opposite to encouraging growth of monospecies biofilms of a specific quality. While there are some comparisons of materials for microalgal production (eg. Christenson and Sims (2012)), it is usually limited to a specific combination of cultivation systems, species and environmental conditions.

Scenario 4: Conversion of different metrics

Biofilm biomass concentration of *Isochrysis* aff. *galbana* (TISO), was measured at different times in three metrics: dry weight (g DW m⁻²), ash-free dry weight (g AFDW m⁻²) and cell count (cells m⁻²). Applications in different industries prefer different metrics for describing biomass concentration. For example in most bulk production contexts, such as biofuels, biomass yields and productivity are expressed based on weight (g, kg) and weight-derived metrics (g m⁻² d⁻¹), respectively. In contrast, in an aquaculture context, microalgae feeds are based on cell numbers or carbon content

(Alajmi and Zeng 2013). When translating research from one context into one another, conversion factors are commonly used to provide approximations, if direct measurements in the right metric are not available. However, microalgal cell composition and size are dependent on cultivation conditions (Huerlimann et al. 2010) and existing conversion factors are often based on older empirical data or are based on simplifications, such as assuming perfectly spherical cells (Reed Mariculture 2015a).

4.4 Materials & Methods

Species used:

Six species were used as mono-cultures, all obtained from the North Queensland Algal Identification/Culturing Facility (NQAIF) at James Cook University in Townsville, Qld, Australia. *Mesotaenium* sp. (NQAIF303), *Scenedesmus quadricauda*. (NQAIF304), *Tetraedron* sp. (NQAIF295), *Desmodesmus armatus* (NQAIF301) where chosens, as these species were isolated in Australia for relevance in waste remediation and bioproducts production (von Alvensleben et al. 2015). *Isochrysis* aff. *galbana* (NQAIF001) and *Prymnesium* sp. (NQAIF005) were chosen due to their relevance as aquaculture feed.

Starter cultures were cultivated in 500 ml of medium in 1 l aerated batch cultures. Bold's basal medium (Nichols and Bold 1965) was used for all species except for *Isochrysis* aff. *galbana* and *Prymnesium sp.*, which were cultivated in L1 medium prepared with filtered seawater (Guillard and Ryther 1962). Starter cultures were grown for one week at 21 °C and 45 μ mol m⁻² s⁻¹ photosynthetically active radiation (PAR).

Agar plate preparation:

The agar plates were prepared with Bold's Basal Medium (Nichols and Bold 1965) for *Mesotaenium* sp., *S. quadricauda, Tetraedron* sp. and *D. armatus* and f/2 marine medium (Guillard and Ryther 1962) for TISO and *Prymnesium* sp.. 20 g l⁻¹ of agar powder (Sigma) were added to standard media and autoclaved at 121 °C for 20 minutes. The hot agar solution was poured into 90 mm or 50 mm Petri-dishes to approximately half the height of the lower plate (~ 10 mm).
Cultivation surface & inoculation:

For scenarios 1 & 2, unprinted newspaper was used as a cultivation surface. Squares (30 mm x 30 mm), were inoculated by simply pipetting 500 μ l of starter culture into a drop at the center of the paper. For scenarios 3 & 4, experiments used 47 mm FG or PVDF filters. The use of these filters allowed to apply a precise amount of culture by vacuum filtration, which allowed for more precise determination of initial biomass.

Cultivation conditions:

The Petri-dishes were placed in small greenhouses (36 cm x 29 cm x 19 cm (l x w x h)) for humidity control. The greenhouses were placed under a bank of cool white fluorescent lights (PHILLIPS TLD 36W/840) for an illumination of 45 μ mol m⁻² s⁻¹ PAR at the biofilm surface, with 12:12 h light regime. The cultivation room was air-conditioned to 21°C. Humidity was maintained as high as possible by placing open container of water in the greenhouses and by misting the air twice a day with a plant mister containing distilled water.

Suspension cultures:

Suspension cultures were prepared in aerated 2 l batch cultures, each containing 1000 ml of Bold's basal medium (Nichols and Bold 1965). The cultures were grown under the same conditions as the biofilm cultures.

Outdoor conditions

The small greenhouses (see above) were placed in a greenhouse in Townsville, QLD, Australia. Light and temperature varied during the day, for an average of 302 μ mol photons m⁻² s⁻¹ and 29.8 °C, but reaching up to 4887 μ mol photons m⁻² s⁻¹ and 35.0 °C during the local afternoon.

Measurements:

Dry biomass was determined gravimetrically after drying the membranes at 105 °C in a microwave asher (Milestone Pyro) for 4h. After 1 h of cooling in a desiccator, the filter was weighted again to determine dry weight (DW). For determining ash-free dry weight (AFDW, glass-fibre filters only), the filters were pre-ashed and tared before the start of the experiments and then further ashed at 500°C (overnight), after determining dry weights.

For determining AFDW of suspension cultures, 40 ml of culture were concentrated by centrifugation (Eppendorf 5810R), the supernatant discarded and the pellet resuspended in 5 ml of distilled water. The suspension was transferred into a 10 ml glass beaker and dried under the same conditions as the biofilms.

Cell counts were determined with a GUAVA flow cytometer (MerckMillipore), following the manufacturers protocol. Algal cells were gated based on forward scatter and red fluorescence signals.

In scenarios 1 & 2, the cultivation area was measured by photographing the Petridishes from directly above and determining the number of pixels covered by biofilm using the 'wand' function in IMAGEJ (http://imagej.nih.gov/ij/). A pipette tip (50 mm long) was placed in the image to provide a reference scale for precise area calculations.

Statistical analysis:

All measurements are reported as the mean of three individual Petri-dishes (or batch systems). Error bars in figures show standard error, but have been omitted in some cases to preserve visual clarity. Significance was tested with a two-tailed Student's t-test (Van Emden 2012), with a threshold of p = 0.05 for statistical significance.

4.5 Results

Scenario 1: Growth behavior of different species and comparison with suspension cultures

With regards to biofilm growth (Figure 4.2)., all four species showed a distinct multiphase (diauxic) growth pattern, with an initial lag phase (day 0 to 3), a period of rapid exponential growth and a second slower growth phase that lasted until the end of the experiment.

Mesotaenium sp. and *S. quadricauda* showed no significant difference in growth, while *D. armatus* grew similar to *Mesotaenium* sp. and *S. quadricauda*, but with a prolonged first growth phase, until day 10. Growth of *Tetraedron sp.* was significantly different, with a steep decline in cell number after inoculation and overall lower cell count, despite slightly higher growth rate between day 7 and 21.



Figure 4.2: Growth curves for biofilms of four different species. The lines indicate the different growth phases, with the corresponding specific growth rates reported in the inserted table.

Figure 4.3 shows the relative growth (Eq. 1) in PDA (solid lines) compared to growth in suspension cultures (dashed lines). Notable are the distinct lag phases in the biofilm cultivation (Day 0 - 3) that are absent in the suspension cultivation. However, biofilms show

Table4.2:SpecificGrowthRatesofsuspension cultures

Specific Growth Rates	d 0 - 7	d 7 - 21
<i>Mesotaenium sp.</i> [d ⁻¹]	0.381	0.099
S. quadricauda. [d ⁻¹]	0.441	0.072
D. armatus. [d ⁻¹]	0.43	0.065
Tetraedron sp. [d ⁻¹]	0.211	0.085

longer exponential growth phases and higher relative growth in the later stages of the cultivation, especially for *S. quadricauda* and *D. armatus*. This is reflected in the specific growth rates of the suspension cultures (Table 4.2), which are lower than those for biofilms during the same periods (Figure 4.2).



Figure 4.3: Relative biomass growth of biofilm (triangles) and suspension (diamonds) cultures.

Calculated as cell count on a given day divided by starting cell count, in cells m⁻² (biofilm) or cells l⁻¹ (suspension), respectively. All y-axes are in natural logarithmic scale. The error bars indicate standard deviation.

Scenario 2: Indoor vs Outdoor conditions

In *Mesotaenium* sp. the move to outdoor conditions showed a distinct effect on the duration of the growth phases and the overall rate of growth (Fig. 4.4). Biofilms in outdoor conditions increased faster reaching stationary phase on day 14, while indoor biofilms were still in exponential phase on day 21.



Figure 4.4: Growth of *Mesotaenium* sp. biofilms under indoor and outdoor conditions.

Error bars indicate standard deviation, The faster growth indicates that the harsher outdoor conditions were not detrimental to growth, despite potentially inhibitory light levels (average of 302 μ mol photons m⁻² s⁻¹, up to 4887 μ mol photons m⁻² s⁻¹) and higher temperatures (29.8 °C, up to 35.0 °C, in February 2013, Townsville Qld, Australia). This indicates that growth estimates obtained under mild indoor conditions might be under-representing the productivity of this biofilm cultivation system in other conditions.

Scenario 3: Growth on different materials

The growth of three different species (*Mesotaenium* sp., *Isochrysis* aff. *galbana*, *Prymnesium* sp.) was compared on two different membrane filter materials, fiberglass and PVDF.



Figure 4.5: Growth of *Mesotaenium, Isochrysis* aff. *galbana* and *Prymnesium* on different substrate materials.

Top: Growth curves of three microalgal species (*Mesotaenium* sp. (*Meso*), *Isochrysis* aff. *galbana* (*Iso*), *Prymnesium sp.* (*Prym*)) on two different substrate materials, fiberglass (FG) and polyvinylidene fluoride (PVDF). Error bars indicate standard deviation.

Bottom: Difference between biomass dry weight on both materials (ΔDW, Eq. 3). Positive values indicate more growth on PVDF, negative values more growth on FG. Error bars indicate standard deviation.

Figure 4.5 shows that there are distinct and significant effects of the material. For *Isochrysis* aff. *galbana*, growth was consistently better on PVDF throughout the

cultivation, while for *Mesotaenium* sp. and *Prymnesium* sp., this was only the case in the early stages of the cultivation (up to day 7).

Scenario 4: Conversion of different metrics

Figure 4.6 shows the growth of an *Isochrysis* aff. *galbana* culture, with the biomass concentration on each day determined as cells counts (left axis, dashed line) and dry weight (right axis, solid line). As indicated by the difference in behavior (cell numbers increase slower than biomass at first, but biomass growth stops earlier), the conversion rate between cell numbers and biofilm biomass (g cell⁻¹ and cell g⁻¹), for was not constant over time (Fig. 4.6), with the highest conversion factor being 2.14 times larger than the lowest one (day 0 and day 7, respectively). This casts doubt on the widespread use of simple multiplications for converting different biomass metrics and highlights the need to consider culture age, cell size in these calculations.





4.6 Discussion:

The presented four scenarios illustrate the use of simple Petri-dish experiments to provide appropriate basic information about biofilm processes and how they can be used to isolate specific factors affecting cultivation. Scenario 1 showed how the differences between species can be small – such as between *Mesotaenium* sp. and *S. quadricauda* – or more pronounced – such as *Tetraedron*. In Scenario 2, the profound difference in growth in indoor and outdoor conditions indicates the need for experiments under realistic conditions. Scenario 3 indicated that the interactions between microalgae and materials is complex and not constant over time – as shown by the difference in behavior between *Isochrysis* aff. *galbana* and the other two species when changing the material. Scenario 4 raises some questions about the use of basic conversion factors and the possibility of systematic bias in reported data.

When investigating a specific application, it is recommended to integrate these questions into a factorial design that tests different combinations of conditions, e.g. 'How will a change of material affect different species at different light levels?' The small scale of the Petri-dish assay is ideal for such experiments, as the large number of individual samples (and replicates) needed over the course of such an experiment can be accommodated on limited space. This allows to account for unexpected interactions and to identify the best combination before moving to larger scale experiments.

The amount of biomass growth is broadly comparable to other work, such as the ones presented in the introduction (Naumann et al. 2012; Liu et al. 2013), despite the much simpler experimental design. Similar growth patterns and cell densities were achieved, for example in Scenario 3, *Isochrysis* aff. *galbana* grown on FG produced 13.8 g DW on day 14, which is almost identical to 14 g on day 16 reported by Naumann et al. (2012).

Despite the small size, the Petri-dish assay can still provide sufficient biomass for more detailed investigation of microalgal biochemical composition. Each filter provides 10 to 15 mg of biomass - depending on conditions or species – which means that pooling a small number of filters yields enough sample material for investigations such as lipid profiles or small feed trials in aquaculture (Table 4.3).

66

Method	Reference	Dried biomass needed	# Filters needed (diameter 47 mm)
Lipid content	von Alvensleben et al. (2013)	30 mg	2 – 3
FAME profile	von Alvensleben et al. (2013)	30 mg	2-3
Protein content	Brown et al. (1997)	4 mg	1
AA profile	Brown et al. (1997)	4 mg	1
Carbohydrates	Brown et al. (1997)	<20 mg	2
Identification by PCR/ Sequencing	von Alvensleben et al. (2015)	< 50 mg	3 - 5
Identification of protein expression (RNA qPCR)	Huerlimann et al. (2014)	< 50 mg	3- 5
Small feed trial (Copepods)	Alajmi and Zeng (2013)	80,000 cells d ⁻¹ per replicate	< 1 filter per replicate per day

Table 4.3: Amount of biomass and filters required for biochemical profiling

While dry weight measurements are common and accurate, they are also a destructive and time-intensive method. Establishing suitable conversion factors and knowing how these factors change, over time or due to cultivation conditions, can greatly benefit experiments and applications. They could also be used to correlate more unusual metrics – such as color change of a biofilm – to biomass, which could enable non-destructive monitoring of biofilm growth.

While not pursued in the presented work, the PDA could also be used to provide samples for more detailed analytical methods, such as measuring photosynthetic activity with PAM fluorometry (Barranguet et al. 2004), detailed depth profiles with microelectrodes or advanced microscopy techniques such as confocal laser scanning microscopy and SEM. Standardized tests would allow for these analytics to occur in different locations and still be comparable across different research groups.

4.7 Conclusion

To conclude, the Petri-dish perfused biofilm assay presents a simple and relatively inexpensive tool for microalgal biofilm research. There is potential for wider application across the field of microalgal biofilm research, in order to achieve a higher degree of standardisation between different research groups. The simplicity of the assay allows for replication and development of factorial experimental designs to begin to reduce the uncertainties in microalgal biofilm research. While outcomes may not be 100% scalable, the PDA offers a platform to at the very least evaluate performance trajectories at a sufficiently replicated level and can be used as a fast and efficient tool to provide initial data to justify investments into larger projects.

Chapter 5: Simulation of biofilm and suspension cultivation processes for the production of microalgal concentrates in aquaculture

5.1 Abstract

Microalgae concentrates are a potential feed for the growing aquaculture industry, providing an alternative to the cost intensive and difficult to maintain on-site cultivation of microalgae in suspension systems. However, there are still open questions regarding the nutritional quality of commercially available concentrates and feed costs are still prohibitively high. One possible alternative to the current production pathways, is the cultivation of microalgal biofilms, which could potentially reduce harvesting effort and result in a cheaper and better quality product.

In this work, a mathematical growth model is employed to simulate an existing *batch suspension cultivation process* in 20 'big bags' and to test three alternative scenarios: Semi-continuous suspension cultivation in 'big bags', as well as batch and semi-continuous biofilm cultivation on vertical panels. The model is formulated to include the necessary cultivation parameters and to allow the simulation of yearly biomass productivity and yearly power consumption for all processes.

The simulation shows that there is considerable benefit in adopting a semi-continuous suspension cultivation strategy. The yearly productivity of the 20 bags in batch suspension mode could be matched by 9 bags in semi-continuous mode, with corresponding improvements in power consumption. In contrast, the biofilm systems showed less productivity per panel than the 'big bags' and consequently required 135 panels in the best case to match the productivity of 20 bags, at much higher power consumption. While this does represent an considerable increase in infrastructure compared to the bag cultivation system, it still shows that the biofilm systems can operate at a comparable scale. As the research into applied biofilm cultivation progresses, there is plenty of room for improvement.

69

Overall, this work demonstrates the suitability of the chosen models. With the appropriate, industrially relevant background data, this simulation could be expanded into a techno-economic analysis to guide future process development.

5.2 Introduction

An increasing proportion of the world-wide seafood is produced in aquaculture, in order to satisfy increasing consumption in view of stagnating or declining wild catches (FAO 2014). Microalgae have been identified as an 'indispensable food source' (Brown et al. 1999) for bivalves and larval stages of fish and crustaceans and are also of interest to replace fish oil (currently still predominantly sourced from wild fisheries) in the feeds for predatory fish, such as salmon (Hemaiswarya et al. 2010; Chauton et al. 2015). However, maintenance of live algae cultures at an aquaculture facility is constrained by the high cultivation costs and limited experience in microalgal cultivation, particularly in smaller farms and hatcheries (Borowitzka 1997).

Microalgal concentrates are an alternative to live algal feeds. In this approach the microalgae are first grown in suspension culture and are then separated from the majority of the cultivation medium at the end of the cultivation, resulting in a thick paste of concentrated algae (Knuckey et al. 2006) with 6 to 8% total solid content (Reed Mariculture 2015a). These concentrates can be packaged, stored at 4 °C and shipped, making microalgae available for facilities without on-site cultivation or as a back-up in case of failure of cultivation systems (Borowitzka 1997; Knuckey et al. 2006). However, the additional processing can damage the algal cells, affecting the nutritional quality of the microalgae and the shelf-life of the concentrates (Tredici et al. 2009). Comparisons between live feeds and concentrates have shown mixed success with the concentrates, sometimes resulting in good performance, sometimes showing inferior growth with the processed product (Brown 2001; Ponis et al. 2003; Hemaiswarya et al. 2010; Aji 2011; Alajmi and Zeng 2013). Furthermore, microalgal concentrates are still expensive, commercially available pastes cost between 40 to 70 USD per kilogram of paste (Reed Mariculture 2015b).

Biofilm cultivation of microalgae is emerging as a potential alternative to suspension cultivation of microalgae. In this approach the microalgae are grown attached to a supporting material with low volumes of liquid medium and as such occur naturally at high cell densities. The cells can be harvested by scraping without the need for further concentration. This approach has been tested for use in aquaculture with a pilot-scale perfused membrane cultivation system (Naumann et al. 2012), showing that

71

production of algae is possible. However, while the species grown are relevant for aquaculture, the biofilms themselves have neither been tested in a feed trial, nor has there been a direct comparison to suspension cultivation. Other approaches have focussed on the cultivation of biofilms for the cultivation molluscs, which directly feed on attached diatoms (Xing et al. 2008).

A large number of innovate biofilm cultivation system are currently emerging, designed for a wide range of applications (reviewed in Berner et al. (2014)², Gross et al. (2015a). In this regard, there is considerable potential for further developments into attached cultivation, including in an aquaculture context. To guide these developments and identify key cultivation parameters, it is beneficial to consider the use of a cultivation system in an applied context of an aquaculture facility. Mathematical models are a convenient tool which allows to assess the effect of different parameter rates as well as changes in equipment or cultivation parameters on the overall process productivity and costs (Borowitzka 2013b).

Process simulations can be developed further into a complete techno-economic analysis, in order to provide a detailed understanding of the economic viability of the process and its commercial potential (Borowitzka 2013b; Dowes and Hu 2013). To successfully achieve this outcome, it is necessary to assess the capital costs (CAPEX) and operating costs (OPEX) for each step of the cultivation process. These costs are combined in a suitable model of sufficient complexity, in a four step cycle of scoping, modelling, evaluating and refining (which leads back to scoping) (Borowitzka 2013b), with the overall aim to determine the general profit of a process and its alternatives (Dowes and Hu 2013). A number of these analyses have recently been described for microalgal fuels (eg. Klein-Marcuschamer et al. (2013) and Nagarajan et al. (2013), and for aquaculture feeds (eg. Suleiman et al. (2014)), although not specifically for the cultivation of microalgae in aquaculture nor specifically for biofilms.

In this work, I address this gap by providing the initial process simulation for biofilm cultivation for the production of microalgal concentrates in aquaculture. For this purpose a dataset produced, under my direct supervision, for an Honours thesis at

² Chapter 2 of this thesis

James Cook University (JCU, Zanoni 2014) is utilised. This data set was generated using the Petri-dish method described in Chapter 4 of this thesis, as a means to investigate the use of the marine microalga *Isochrysis* affinis *galbana* (TISO) for the cultivation of the tropical calanoid copepod *Parvocalanus crassirostris*. The detailed methods are described in Appendix 5.2. This was a continuation of prior work (Alajmi and Zeng 2013) into use of microalgal concentrates for this feeder organism in aquaculture. For this purpose, TISO biofilms and suspension cultures were grown under identical conditions and eventually used in a feed trial. This research showed that biofilms preformed as well as live feeds in this context and considerably better than commercially available concentrates (Zanoni 2014).

To provide a realistic applied context, the scope of the production is based on the cultivation process used at an Australian aquaculture hatchery, FinFishEnterprise (FFE) in Cairns, QLD. This hatchery is currently cultivating live TISO for use as aquaculture feed, although without a concentration step, as the microalgal culture is fed directly into the feeder tanks, rather than processed to a paste. The microalgae are grown in an indoor facility under artificial light in so-called 'Big Bags'; disposable, cylindrical polyethylene bags that are supported by a mesh frame and mixed by constant aeration (Tredici et al. 2009; FinFishEnterprise 2015). The process currently in use is expanded to include a concentration step (centrifugation) and the MATLAB software package (MathWorks) is used to simulate the production of microalgal paste and quantify productivity and power costs of the process. In addition, three alternative cultivation scenarios are compared: Semi-continuous cultivation in suspension and biofilm cultivation, both as batch and semi-continuous process.

This work does not contain a full techno-economic analysis, as there is still considerable uncertainty in process OPEX and CAPEX, due to the large scale up factor between the available laboratory experiments and production scale. In this work, the first model for microalgal biofilm growth has been designed to be applied to biomass production in microalgal aquaculture. This serves the purpose of the development of an initial framework, suitable for an applied question from industry. However, this framework can also assist future refinement and direct future research and data

73

generation, which can then be used to further refine the theoretical work and advance knowledge.

The overall structure of the chapter is based on the process outlined in Borowitzka (2013b) of 1. Scope definition, 2. Model description and 3. Evaluation of the results. An outlook on possible refinement is included in the last section, 4. Conclusions.

5.3 Scope definition

The overall scope of this simulation is to characterise the production of concentrated microalgae paste of the species TISO at a small to medium enterprise (SME). In the calculations that follow this production process is conceptualised in four processing steps (Figure 5.1):

- 1. Inoculation, in which the system is set-up and the initial conditions for the growth step are defined.
- 2. Growth, in which the microalgae biomass increased. This is the express purpose of this bio-production system and as such one of the key process steps.
- Harvesting, in which the microalgae are removed from the system and dewatered. This is another key step, as the actual product, microalgal concentrate, is created.
- 4. System reset, in which the system is restored to its original state, ready for inoculation. This is the prerequisite step to initiate another growth cycle.

Process flow diagram



Figure 5.1: Overall process flow for microalgal cultivation, showing the 4 processing steps.

Regrowth only occurs in the semi-continuous production scenarios, not the batch scenarios. The grey processing steps are not included in the simulation.

Processing steps further upstream and downstream of these steps are excluded from the simulation. For example, excluded are preparation of the culture and cultivation medium (upstream) and further processing and packaging of the concentrated microalgae after harvesting/dewatering (downstream processing). Including these steps would greatly increase the complexity of the simulations, as they are multi-step processes with several possible alternative approaches

TISO was chosen as it is a relevant species for aquaculture, including at FFE, and because of the availability of a complete matching dataset of growth in suspension and biofilm culture, under identical conditions.

The cultivation process is first simulated as batch suspension cultivation based on the process currently in use at FFE, with the addition of a harvesting step. The overall biomass productivity and, as an example OPEX, power costs for inoculation, lights and harvesting are simulated and serve as a baseline for three alternative scenarios, scaled to match the productivity of that process:

- 1. Suspension cultivation in semi-continuous mode
- 2. Biofilm cultivation in batch mode
- 3. Biofilm cultivation in semi-continuous mode.

5.4 Numerical approach

MATLAB was used to solve the model equations. In the code, all process states and costs are represented as arrays with process variables organised using time in columns

and replicate system variables in rows. For example, the biomass in a system of 20 bags that are cultivated over 25 days is contained in a 20 x 25 matrix with the initial biomass in the first column, the biomass for the next day in the second column etc. and with each row representing a different bag. Array elements were summed to determine overall cultivation parameters – for example the sum of all rows in a given column would result in the total biomass in all bags. Process operations such as harvesting are also tracked in time-dependent arrays. The code used for this work is provided in Appendix 5.4

5.5 Model description

The approach used for the simulation is described using the baseline process of the batch suspension cultivation. Relevant concepts, equations and key inputs which establish the overall scale of production are presented. An overview of the results is provided to define the basis for the three alternative scenarios. Each scenario is introduced in sequence, detailing any modifications to the baseline model and inputs, starting with the semi-continuous suspension cultivation, followed by the batch biofilm cultivation and finishing with the semi-continuous biofilm cultivation.

The model describes the production of biomass over time, with repeated cycles of inoculation, growth and harvesting (Eq. 5.2ff) The simplicity of the model is intentional because of the substantial uncertainty in both details in the mechanisms of biofilm growth and the parameters governing performance. The model was developed using available data available data from FFE to empirically determine necessary growth rates and, where necessary, utilises data determined through prior experiments at JCU. Despite the relatively simplicity of the model, it is suitable for the use in a techno-economical analysis. Such studies - at least initially - aim to provide a broad overview of the while process and are consequently based on estimates of numerous process options (Borowitzka 2013b; Dowes and Hu 2013). In this regard, the model is sufficiently complex to provide a quantitative overview of microalgal growth, the determining factors when developing business cases for industrial-scale production processes Furthermore, since this model seeks to compare traditional microalgal suspension cultivation systems with biofilm-based production systems, quantification of the energy costs of centrifugation is critical.. More sophisticated refined models for

76

biofilm cultivation have been described (eg. Wolf et al. (2007), Liao et al. (2012), Cumsille et al. (2014) and Munoz Sierra et al. (2014)). However, these models will require additional data that is rarely available for the systems and species of interest, reinforcing earlier conclusions regarding standardisation (see Chapter 2).

Artifical light makes up a large proportion of the actual production cost (FinFishEnterprise 2015). Centrifugation is the standard method for dewatering suspension cultures in order to produce microalgal paste, representing a very energy-intesive step (Molina Grima et al. 2003). As this step is eliminated in biofilm cultivation, Comparison of these energy costs is essential.

A glossary of the variables used is provided in Appendix 5.1. The details of the methods used to generate the microalgal growth data are described in Appendix 5.2 and the detailed calculations to estimate and justify the power consumption equations for the various process steps are described in Appendix 5.3.

Baseline process description: Batch suspension cultivation

Overview

The suspension system is conceptualised as a number of large, cylindrical bags, in which the microalgae are grown as free-swimming cells in liquid medium. The bags are located indoors and are illuminated by light panels from both sides (Figure 5.2). Cultivation occurs in a batch process, where all the cultivation volume is harvested/dewatered at one time, in order to produce concentrated microalgae paste. A number of batches are run in sequence, over the course of a year. The concept and overall size of the system is based on the cultivation process at FFE; the specific cultivation conditions, growth performance and light intensity are based on the conditions and lamps used in the laboratory experiments.

System size

The cultivation process at FFE (FinFishEnterprise 2015) consists of an indoor facility with 20 individual 'Big Bags', each with a volume of 500 l. This defines the overall scale of the system, with the number of bags N_{BAG} = 20 and the volume of each bag V_{BAG} = 500 l.

77



Figure 5.2: Schematic overview of the microalgal bag cultivation system.

The system consists of number of cylindrical bags (green), illuminated (on both sides) by banks of fluorescent lights (yellow).

Cultivation start & inoculation

The cultivation follows a batch growth mode. The cultivation starts at a defined initial biomass concentration, which is created by adding a previously prepared inoculation culture, with a defined biomass concentration of X_{INOC} [g Γ^1] = 0.6 g Γ^1 (dry weight (DW) – all biomass in this work is expressed in dry weight) to each bag and filling up the remainder of the volume with cultivation medium. The numerical values for the growth model are based on experimental data (Figure 5.3, adapted from Zanoni (2014)), which provides the values for all relevant metrics. This defines the starting biomass concentration for each bag X_{0BAG} 0.0625 g Γ^1 and allows to calculate the volume of inoculation culture, $V_{INOCBAG}$ needed for each bag as

$$V_{INOCBAG} = \frac{V_{BAG} * X_{0BAG}}{X_{INOC}}$$
(Eq. 5.1)

The inoculum culture has to be cultivated before the start of the cultivation and as such has occurred a power cost. This defines the power consumed for the preparation of the inoculum as $P_{INOC} = 0.117$ KWh I^{-1} .

Filling the bags at the start of the cultivation requires some pumping, which defines the power consumed for filling the bags $P_{FILLBAG} = 5.8 * 10^{-6} \text{ KWh I}^{-1}$



Figure 5.3: Growth curve of Isochrysis aff. galbana (TISO, NQAIF 001) suspension cultures in f/2 (Guillard and Ryther 1962; Guillard 1975).

Overlay of data (diamonds) from Zanoni (2014) with fitted specific growth rates μ [d⁻¹] (lines), for each growth phase (day 0 – 7, day 7 – 28), used in the simulation. The y-axis is in logarithmic scale to show linear growth rates. See appendix 5.2 for details on the cultivation methods.

Microalgal growth

This gives each bag an initial concentration of biomass, which increases over the course of a number of days until a predetermined harvesting time. This growth follows the typical pattern for exponential microbial growth (Becker 1994) and can be described as follows:

$$X_2 = X_1 * e^{\mu_t (t_2 - t_1)}$$
(Eq. 5.2)

This relates the biomass concentration at a later time X_2 to the biomass concentration at an earlier time X_1 multiplied by an exponential term with the specific growth rate during that time μ_t multiplied by the difference between time t_2 and time t_1 . μ_t is determined as the slope of a linear regression fitted to the natural logarithm of the biomass concentration X versus time t in a semi-logarithmic plot.

$$\frac{\ln(X_2)}{\ln(X_1)} = \ln(X_2 - X_1) = \mu_t(t_2 - t_1)$$
(Eq. 5.3)

This was applied to the experimental data in Figure 5.3, to determine μ for the two growth phases, defining μ_1 (for day 0 – 7) = 0.3231 d⁻¹ and μ_2 (day 7 – 25) = 0.0532 d⁻¹.

During the cultivation, the bags are continuously aerated, which defines the power used for aeration $P_{AERBAG} = 0.0026$ KWh l⁻¹ d⁻¹. Cultivation occurs under artificial lights and each bag is assumed to be illuminated 12h a day by 14 TL-D 36W/840 1PP fluorescent lamps. This defines the variables for power consumption of the artificial lights, with the duration of illumination $T_{LAMPBAG} = 0.5$, the number of lamps per bag $N_{LAMPBAG} = 14$ and the power consumed by each lamp $P_{LAMP} = 1.008$ KWh d⁻¹.

In the actual computation, Eq. 2 is used to calculate the biomass growth for each day based on the biomass concentration of previous day. This calculation is repeated to track the increase in biomass concentration over the duration of the batch ($T_{BATCHBAG}$) at which point the culture is harvested. The model calculations are repeated for every possible duration of $T_{BATCHBAG}$ (1 – 28 d, limited by the available data) in order to find the optimal biomass productivity (see below) – the reasons for choosing to do so are explained at the end of this section, as it involves several parameters not yet introduced.

Harvesting

At the end of the batch, all of the culture is harvested from the bags and concentrated to a paste. The process parameters for this step are based on an Evodos 25 centrifuge (Evodos 2015), which is designed specifically for the concentration of microalgal cultures. The harvesting step defines the volume harvested from each bag $V_{HARVBAG}$ = 500 l which is equal to V_{BAG} , as there are no changes in volume during batch cultivation. The overall biomass harvested from each bag $B_{HARVBAG}$ [g] can be calculated

80

from the volume harvested $V_{HARVBAG}$ and the biomass concentration at the time of harvesting X_t which is known from the solution of Eq. 2 for $T_{BATCHBAG}$.

$B_{HARVBAG} = V_{HARVBAG} * X_{T_{BATCHBAG}}$ (Eq. 5.4)

Operating the centrifuge consumes energy, in particular for the separation process itself and for the discharge of the concentrated paste, according to manufacturer information (Evodos 2015). This defines the Power consumption for the separation per volume of culture processed $P_{SEPBAG} = 1.2 * 10^{-3}$ KWh l⁻¹ and the power consumption for the discharge of the paste per amount of paste produced $P_{DISCBAG} = 2 * 10^{-5}$ KWh g⁻¹

Resetting the system

After centrifugation, the bags are dismantled and disposed and new bags are prepared, ready to be filled and re-inoculated; this final step is collectively called 'system reset'. The time needed for these procedures defines $T_{RESET} = 2$ days based on personal laboratory experience.

Yearly productivities and costs

The total duration modelled was one year, $T_{TOT} = 365$ d, resulting in the yearly biomass productivity YB_{BAG} [kg yr⁻¹] and the yearly power consumption YP_{BAG} [KWh yr⁻¹]. The quantities are defined Eq. 7 and Eq. 9, respectively.

Each repeat of the process from inoculation to end of system reset is called one cultivation cycle. Each of these cycles lasts a number of days, defined as cycle time T_{CYCBAG} [d], which is calculated as the sum of the duration of the batch $T_{BATCHBAG}$ and the time needed for system reset T_{RESET} .

$$T_{CYCBAG} = T_{BATCHBAG} + T_{RESET}$$
(Eq. 5.5)

The number of cycles that are possible over a course of a year, N_{CYCBAG} [yr⁻¹], which is calculated by dividing the total time T_{TOT} by the length of each cultivation cycle T_{CYCBAG} , rounding down as only full cultivation cycles are considered.

$$N_{CYCBAG} = \frac{T_{TOT}}{T_{CYCBAG}}$$
(rounded down) (Eq. 5.6)

The yearly biomass productivity YB_{BAG} [kg yr⁻¹] is calculated by multiplying the amount of biomass harvested from one bag $B_{HARVBAG}$ by the number of bags in the system N_{BAG} and the number of cultivation cycles N_{CYC} and divided by 1000 to convert from g to kg.

$$YB_{BAG} = \frac{B_{HARVBAG} * N_{BAG} * N_{CYCBAG}}{1000}$$
(Eq. 5.7)

The yearly power consumption YP_{BAG} [KWh yr⁻¹], is estimated by first calculating the power consumption for each cultivation cycle, defined as P_{BAG} [KWh], as the sum of the power consumed for each process step: Preparation of the inoculums (P_{Inoc}) and filling of the bag ($P_{FILLBAG}$) during inoculation, aeration (P_{AERBAG}) and illumination ($N_{LAMPBAG}$, P_{LAMP} , $T_{LAMPBAG}$) and the energy used to run an Evodos 25 centrifuge during harvesting (P_{SEPBAG} , $P_{DISCBAG}$).

$$P_{BAG} = P_{INOC} * V_{INOCBAG} + P_{FILLBAG} * V_{BAG} +$$
Inoculation (Eq. 5.8)

$$(P_{AERBAG} + N_{LAMPBAG} * P_{LAMP} * T_{LAMPBAG}) * T_{CYC} +$$
Cultivation

$$P_{SEPBAG} * V_{HARVBAG} + P_{DISCBAG} * B_{HARVBAG}$$
Harvesting

Multiplication of this value by the number of bags N_{BAG} and the number of cultivation cycles N_{CYC} results in the yearly power consumption YP_{BAG}

$$YP_{BAG} = P_{BAG} * N_{BAG} * N_{CYCBAG}$$
(Eq. 5.9)

In addition to the overall power consumption, it is also of interest to know how much power is needed for the production of a given amount of biomass, which defines the specific power consumption SP_{BAG} [KWh kg⁻¹]:

$$SP_{BAG} = \frac{YP_{BAG}}{YB_{BAG}}$$
(Eq. 5.10)

Optimal value of *TCYC* **+** *TBATCHBAG*

The overall duration of each cultivation cycle (T_{CYC} , Eq. 5.5) is directly linked to the duration of the growth phase ($T_{BATCHBAG}$), which has a key role in the cultivation process. An increase $T_{BATCHBAG}$ results in an increase in harvested biomass $B_{HARVBAG}$, since the cell concentration in the system increases over time, but also reduces the number of cultivation cycles per year (N_{CYC}), as each cultivation cycle T_{CYC} is longer. Consequently a longer $T_{BATCHBAG}$ results in fewer harvests per year, but with more

biomass per harvest, and a shorter $T_{BATCHBAG}$ results in more harvests per year but at lower biomass. As such there is an optimal value for $T_{BATCHBAG}$ which results in the highest possible yearly biomass productivity (YB_{BAG}).

Similarly, $T_{BATCHBAG}$ influences the yearly power consumption YP_{BAG}, (via Eq. 5.8, 5.9 & 5.10). However, for the purpose of this work, the basis for comparison is the yearly biomass productivity YB_{BAG}.

Results of baseline suspension batch cultivation

Figure 5.4 illustrates the results for yearly biomass productivity YB_{BAG} and yearly power consumption YP_{BAG} , as a function of the overall cycle time, T_{CYC} . The best yearly productivity is achieved for a duration of the cultivation cycle (T_{CYC}) of 9 days, corresponding to, $T_{BATCHBAG}$ = 7 days. Over the course of 40 cultivation cycles (N_{CYC} = 40) in a year, this results in a yearly biomass productivity YB_{BAG} of 240 kg yr⁻¹ (dry weight) and a yearly power consumption YP_{BAG} of 52156 KWh yr⁻¹ and a specific power consumption of SP_{BAG} of 217.3 KWh kg⁻¹. This is also the lowest specific power consumption (data not shown).



Figure 5.4: Results of the baseline batch cultivation process

Yearly biomass productivity (YB_{BAG}) (top) and total power consumption (YP_{BAG}) (middle) and specific power consumption (SP_{BAG}) (bottom) for the batch suspension microalgal cultivation process, versus duration of the cultivation cycle (T_{CYC}).

Alternative production scenarios:

Modelling of the alternative production scenarios follows the same principles as the baseline process, but without assigning a specific number of cultivation systems (bags or panels) beforehand. Instead, biomass production is calculated for a single system and the result is used to calculate the number of systems needed to match the yearly productivity of the baseline process, $YB_{BAG} = 240 \text{ kg yr}^{-1}$. This approach was chosen to guarantee that the comparisons between suspension and biofilm systems would be

valid for the fundamental objective of biomass production, as the systems do use slightly different metrics and growth models.

Scenario 1: Semi-continuous suspension cultivation

Overview

The semi-continuous suspension process (SCB) uses the same fundamental models as the batch cultivation, unless otherwise noted. The main difference is in notation, as the indicator '...BAG' is replaced by '...SCB', where appropriate, eg. N_{CYCBAG} becomes N_{CYCSCB} and YB_{BAG} becomes YB_{SCB}.

The semi-continuous suspension cultivation is conceptualised to use the same equipment as the batch cultivation, consisting of a number of bags and the necessary processing infrastructure. The process starts out identically to the batch cultivation, starting with inoculation and growth until $T_{BATCHSCB}$, using the same growth model (Figure 5.2) At this point, the two approaches diverge: Rather than harvesting all of the biomass (with a concentration of $X_{TBATCHSCB}$), only part of the culture volume in each bag is processed and concentrated. The rest of the volume remains in the bag, fresh nutrient medium is added and the remaining, diluted, biomass is regrown for one or more days (T_{RGSCB}) back to the concentration $X_{TBATCHSCB}$. The biomass is harvested again and the regrowth is repeated a number of times.

Cultivation cycle

An example for this growth pattern is shown in Figure 5.5, for a $T_{BATCHSCB}$ = 12 d and a T_{RGSCB} = 8 d, overlaid over the experimental growth data.

As with $T_{BATCHBAG}$ in the baseline scenario, the combination of $T_{BATCHSCB}$ and T_{RGSCB} affects the harvested biomass concentration, the duration and number of cultivation cycles and the power consumption. A shorter $T_{BATCHSCB}$ allows for earlier harvesting and more cultivation cycles but at reduced biomass concentration. A shorter T_{RGSCB} allows for more frequent harvesting cycles, but with lower volume and biomass productivity for each cycle.



Figure 5.5 Microalgal biomass concentrations in a semi-continuous bag cultivation process, ($T_{BATCHSCB} = 12 d and$, $T_{RGSCB} = 8 d$) versus the experimental growth data. Overlay of the semi-continuous growth track (dark line) versus the experimental data (diamonds) and the batch growth track (thin line).

Due to age and accumulation of waste products, this regrowth can only be repeated for a limited duration, after which the microalgal culture becomes too unstable for further cultivation. At this point all the biomass is harvested, old bags are removed and new bags are prepared, to reset the system. This maximal duration is defined as $T_{MAXRGSCB} = 60$ d. This is aPM conservative estimate, as TISO cultures have been maintained up to twelve months in continuous systems (Marchetti et al. 2012).

Figure 5.6 shows the mathematical simulation of the biomass concentration over the whole cultivation cycle, for $T_{BATCHSCB} = 12$ d and, $T_{RGSCB} = 8$ d.



Figure 5.6: Simulation of the microalgal biomass concentration in the semicontinuous microalgal bag cultivation process, for $T_{BATCHSCB} = 12 d$ and, $T_{RGSCB} = 4 d$. The biomass concentration (circles) is tracked over time. The dotted lines illustrate how the biomass grows up to $X_{TBATCHSCB}$ and is then harvested back to $X_{TBATCHSCB-TRGSCB}$, the concentration 8 days earlier. This occurs every T_{RGSCB} days, for duration of $T_{MAXRGSCB}$.

The number of regrowth cycles, defined N_{CYCRG} can be calculated by dividing $T_{MAXRGSCB}$ by T_{RGSCB} , rounded down to only allow full cycles.

$$N_{CYCRG} = \frac{T_{MAXRGSCB}}{T_{RGSCB}}$$
(rounded down) (Eq. 5.11)

As in the batch process, the system is reset to original state at the end of the cultivation, which defines the overall length of the full cultivation cycle T_{CYC} [d], consisting of batch phase and a number of regrowth cycles:

$$T_{CYCSCB} = T_{BATCHSCB} + N_{CYCRG} * T_{RGSCB} + T_{RESET}$$
(Eq. 5.12)

Harvesting

To facilitate modelling, T_{RGSCB} is constrained to full days (up to T_{BATCH}) with regrowth occurring at the same μ as during the initial batch cultivation. It follows, that in order for the biomass to regrow to identical concentration X_{TBATCH} , it must be harvested to the concentration that it was T_{RGSCB} days ago equal to $X_{TBATCH-TRGSCB}$. This facilitates the calculation of the volume harvested ($V_{HARVESTED}$ [I]) as a proportion of the total volume of each bag:

$$V_{HARVSCB} = \left(1 - \frac{X_{TBATCH-TRGSCB}}{X_{TBATCH}}\right) * V_{BAG}$$
(Eq. 5.13)

The amount of biomass harvested, B_{HARVSCB} for each regrowth cycle can be calculated from the current biomass concentration and the volume harvested.

$$B_{HARVSCB} = X_{TBATCH} * V_{HARVESTED}$$
(Eq. 5.14)

Yearly productivities and costs

As in the batch process, the cultivation cycle is repeated a number of times (N_{CYC}) over the duration of a year. Unlike the batch process, incomplete cycles are considered as well, as cultivation cycles can still result in harvested biomass.

The yearly biomass production YB_{SCB} is calculated for a single system only

$$YB_{SCB} = B_{HARVSCB} * N_{CYCSCB}$$
(Eq. 5.15)

This value is then used to determine the number of bags needed, defined as N_{SCB} , rounded up to the nearest full bag – as such the yearly production of the semicontinuous system will minimally exceed the yearly production of the baseline cultivation.

$$N_{SCB} = \frac{YB_{BAG}}{YB_{SCB}}$$
(rounded up) (Eq. 5.16)

 N_{SCB} was calculated for every possible combination of the combination of $T_{BATCHSCB}$ and T_{RGSCB} to determine the best combination with the lowest possible number of bags.

Scenario 2: Biofilm batch cultivation

Overview

The biofilm system is conceptualised to consist of an array vertical panels in an indoor cultivation space (Figure 5.7). Each panel consists of a central liquid conducting layer, covered on each side with a cultivation surface, consisting of a cell impermeable membrane, on which the biofilm grows; see Figure 5.8 for a cross-section of a panel. Each side of each panel is illuminated by a bank of lights. Cultivation medium is applied at the top of the liquid conducting layer und is collected at the bottom, from where it is pumped back to the top.

The specific cultivation conditions, growth performance and light intensity are based on the conditions and lamps used in the laboratory experiments. With regards to notation, the indicator $'_{\dots PAN}$ ' is used to refer to parameters for this scenario.



Figure 5.7: Schematic overview of the PMBR panel cultivation system

The system consists of number of panels (green), illuminated (on both sides) by banks of fluorescent lights (yellow).

The cultivation process starts with inoculation, by vacuum filtering the (liquid) inoculation culture onto the semi-permeable membranes. The membranes are placed on the panels and the biofilm grows until the day of harvest, when all the biomass is removed by manually scraping it off the surface. The panels are dismantled and fresh panels are prepared, ready for another cultivation cycle. Essentially this process is identical to the baseline batch suspension process, with the Big Bags replaced by biofilm panels.



Figure 5.8: Operating principle of perfused membrane photo-biofilm reactors

The microalgal biofilm (green) grows on a semi-permeable membrane (horizontal hatching) on top of a liquid conducting base layer (blue crosshatching). The microalgae are directly exposed to light and CO₂. The membrane allows the passage of water and nutrients, but prevents the cells from passing into the base layer, illustrated by the dark green ellipses and the red crossed arrows.

(Repetition of Figure 3.1, to illustrate the explanations in this chapter in context)

System size

The cultivation process consists of an indoor facility with a number of individual biofilm panels (Figure 5.7), each with a cultivation area on 2 m² (1m² on each side). This defines the overall size of the panels, with the cultivation area of each panel $A_{PAN} = 2 \text{ m}^2$. The number of panels N_{PAN} is an output variable of the model.

Cultivation start & inoculation

The cultivation follows a batch cultivation model, with a defined initial biomass concentration. The cultivation model is based on experimental data (Figure 5.9, after Zanoni (2014)), which provides the values for all relevant metrics. Data was available for two different surface materials, fibre glass (FG) and polyvinylidene fluoride (PVDF), each with different inoculation and growth parameters. The process is simulated for both materials, with parameters specific to one material being distinguished the subscripts ...FG or ...PVDF. E.g. the number of FG panels needed would be N_{PANFG}.



Figure 5.9: Growth curve of *Isochrysis aff. galbana* (TISO) biofilms on different materials Overlay of data (dots & squares) from Zanoni (2014) with fitted specific growth rates μ [d⁻¹] (lines), for each growth phase (day 0-3, day 3 – 10, day 10 - 28), as used in the simulation. The y-axis is in logarithmic scale to show linear growth rates.

The cultivation data defines the starting biomass concentration for each panel, with $X_{OPANFG} = 18.84 \text{ g m}^{-2}$ and $X_{OPANPVDF} = 7.31 \text{ g m}^{-2}$. The volume of inoculation culture $V_{INOCPAN}$ needed for each panel is calculated as:

$$V_{INOCPAN} = \frac{A_{PAN} * X_{0PAN}}{X_{INOC}}$$
(Eq. 5.17)

The inoculum culture has to be cultivated before the start of the cultivation and as such has occurred a power cost. The power consumption is again defined as $P_{INOC} = 0.117 \text{ KWh I}^{-1}$.

The vacuum filtration also requires some power, which is defined as $P_{VACPAN} = 1.0*10^{-4}$ KWh per litre of inoculation culture.

Microalgal growth

The inoculation provides each panel an initial concentration of biomass, which increases over the course of a number of days until a predetermined harvesting time. This growth follows a similar pattern as observed in the baseline process – with an added initial lag phase, which is followed by two growth phases (Figure 5.9). Biomass concentration is expressed per cultivation surface area [g m⁻²] rather than volume [g l⁻¹]. The experimental data defines the specific growth rates, as per table 5.1:

	FG	PVDF
μ ₁ (day 0 – 3)	0 d ⁻¹	0 d ⁻¹
μ ₂ (day 3 – 10)	0.0666 d ⁻¹	0.1428 d ⁻¹
μ ₃ (day 10 – 28)	0.0191 d ⁻¹	0.0211 d ⁻¹

Table 5.1 Specific growth rates for biofilm cultivation

During the cultivation, the liquid medium needs to be recirculated to the top of the liquid conducting layer, which requires pumping power ($P_{RECPAN} = 0.4205$ KWh m⁻² d⁻¹). Cultivation occurs under the same light intensity as in the baseline process and each side of the panels is assumed to be illuminated 12h a day by seven 36W fluorescent lamps, each with a nominal power consumption of 1.008 KWh d⁻¹.

As in the baseline process, the model calculations are repeated for every possible duration of cultivation, $T_{BATCHPAN}$ (1 – 28 d, limited by the available data) in order to match the optimal biomass productivity of the baseline batch suspension system.

Harvesting

At the end of the batch phase all of the biofilm is harvested from the panels. This is achieved by manually scraping the biofilm off the cultivation surfaces which directly results in concentrated paste. The whole biofilms area is harvested in every case, defining the area harvested per panel as $A_{HARVPAN} = A_{PAN} = 2 \text{ m}^2$. The overall biomass $B_{HARVPAN}$ [g] can be calculated from the area harvested $A_{HARVPAN}$ and the biomass concentration at the time of harvesting X_t which is known from the solution of the growth equations for $T_{BATCHPAN}$.

$$B_{HARVPAN} = A_{HARVPAN} * X_{t=T_{BATCHPAN}}$$
(Eq. 5.18)

No power costs are incurred during harvesting, as it is assumed to be an entirely manual process.

Resetting the system

After harvesting the panels are cleaned and fitted with new cultivation membranes, ready to be re-inoculated, i.e. the system is reset to its initial state. The time needed for these procedures is assumed to be the same as for the baseline batch suspension process, with $T_{RESET} = 2$ days.

Annual productivities and costs

The duration of each cultivation cycle (T_{CYCPAN}) and the number of cycles (N_{CYCPAN}) are calculated as for the baseline batch suspension process.

$$T_{CYCPAN} = T_{BATCHPAN} + T_{RESET}$$
(Eq. 5.19)

$$N_{CYCPAN} = \frac{T_{TOT}}{T_{CYCPAN}}$$
(rounded down) (Eq. 5.20)

The yearly biomass productivity YB_{PAN} [g yr⁻¹] is calculated as in scenario 1, as the number of cultivation systems is not yet known:

$$YB_{PAN} = B_{HARVBPAN} * N_{CYCPAN}$$
(Eq. 5.21)

Which allows to calculate the number of panels needed, defined as N_{PAN} to match the yearly production in the baseline cultivation, again rounded up the nearest full panel.

$$N_{PAN} = \frac{YB_{PAN}}{YB_{SCB}}$$
(rounded up) (Eq. 5.22)

The power consumption for each cultivation cycle, defined as P_{PAN} [KWh], is determined by summing up the power consumed during the inoculation and cultivation step:

$$P_{PAN} = (P_{INOC} + P_{VACPAN}) * V_{INOCBAG} +$$
Inoculation (Eq. 5.23)

$$(P_{RECPAN} + N_{LAMPBAG} * P_{LAMP} * T_{LAMPBAG}) * A_{PAN} * T_{CYC}$$
 Cultivation

This allows to calculate the total yearly power consumption YP_{PAN} and the specific power consumption SP_{PAN} per Eq. 5.9 & Eq. 5.10.

As in the baseline scenario, the duration of $T_{BATCHPAN}$ affects the harvested biomass concentration, the number of cultivation cycles and the power consumption. Using the same approach as in Scenario 1, the model was run with value of $T_{BATCHPAN}$ (1 - 28 d) and in order to find the conditions resulting in minimal number of systems N_{PAN}.

Scenario 3: Biofilm semi-continuous cultivation

Overview

The semi-continuous biofilm system (SCP) is conceptualised as a number of vertical panels upon which biofilm is grown in semi-continuous cultivation mode. It is, in essence, a combination of the approach used for the semi-continuous suspension system in scenario 1, applied to the equipment, procedures and growth data of the batch biofilm system in scenario 2.

The usual changes in notation apply, with the indicators $'_{...SCB}$ and $'_{...PAN}$ being replaced by $'_{...SCP}$, where appropriate, eg. $X_{OPANPVDF}$ becomes $X_{OSCPPVDF}$.

System size, inoculation and batch growth

This follows exactly the processes described in Scenario 2, for both materials, until the end of batch cultivation, T_{BATCHSCP}.

Regrowth

Regrowth in the semi-continuous biofilm systems is based on the same concepts as in the semi-continuous suspension system and follows the same growth pattern, dependent on the duration of the batch phase $T_{BATCHSCP}$, and the duration of the regrowth T_{RGSCP} . The maximum duration for regrowth is also defined as $T_{MAXRGSCP} = 60$
d. The number of regrowth cycles (N_{CYCR}) and the duration of the overall cultivation T_{CYCSCP} are calculated as per Eq. 5.10 & 5.11.

A minor change is required with regards to harvesting. In the semicontinuous biofilm system, the whole cultivation surface (with a biomass concentration of $X_{TBATCHSCP}$ is scraped at every harvest, but harvesting does not remove all the biomass. A proportion of the biomass (at a concentration $X_{TBATCHSCP}$) remains to act as the initial biomass for regrowth. As such, only the concentration of the biomass per surface area changes, but not the surface area itself.

The amount of biomass harvested $B_{HARVSCP}$ [g] can be calculated directly from these concentrations and the surface area of the system.

$$B_{HARVSCP} = (X_{TBATCH} - X_{(TBATCH - TRGSCB)}) * A_{PAN}$$
(Eq. 5.24)

To facilitate modelling, as in the semicontinuous suspension system, T_{RGSCP} is constrained to full days (up to T_{BATCH}) with regrowth occurring at the same μ as during the biofilm batch cultivation. It follows, that in order for the biomass to regrow to identical concentration X_{TBATCH} , it must be harvested to the concentration that it was T_{RGSCP} days ago equal to $X_{(TBATCH-TRGSCP)}$

From $B_{HARVSCP}$, the overall yearly biomass productivity YB_{SCP} , the number of panels required N_{SCP} , the yearly power consumption YP_{SCP} and the specific power consumption can be calculated using Eq. 18 – 20 and Eq. 9 & Eq. 10, respectively.

As for the semi-continuous suspension system, N_{SCB} was calculated for every possible combination of the combination of $T_{BATCHSCP}$ and T_{RGSCP} to determine the best combination with the lowest possible number of panels to match the production of the baseline batch suspension.

5.5 Evaluation & Results

Model validation

The model would ideally be validated against biomass productivities from comparable systems from the literature. However, there is a distinct lack of reliable information regarding the production of TISO in big bag systems, despite their apparent popularity – available publications focus either on other more advanced cultivation systems or on

small-scale investigations into metabolic pathways and feed optimisation. Information from the aquaculture facilities themselves remains unpublished. The best available reference is from Tredici et al. (2009), stating that volumetric productivities in vertical column systems are "typically below 0.1 g $I^{-1} d^{-1}$ ".

The volumetric productivity VP_{BAG} [g $I^{-1} d^{-1}$] for the baseline batch suspension process can be calculated from the annual productivity YB_{BAG} , the volume and number of bags (V_{BAG} , N_{BAG}) and the number of productive days per year ($T_{BATCHBAG}$, N_{CYCBAG}):

$$VP_{BAG} = \frac{YB_{BAG}}{V_{Bag} * N_{BAG} * T_{BATCHBAG} * N_{CYCBAG}} = 0.0857 \text{ g} \text{ l}^{-1} \text{ d}^{-1}$$
(Eq. 5.25)

Although limited by the available literature, this does indicate that the growth model is valid and delivers comparable outputs to aquaculture systems.

For the biofilm batch simulation (Scenario 2), Naumann et al. (2012) provide data at pilot-scale for biofilm cultivation of TISO, although under outdoor conditions and on unprinted newspaper as surface material. Areal productivity of 0.6 g m⁻² d⁻¹ per cultivation surface area, over a period of 10 days is reported. The areal productivity AP_{PAN} [g m⁻² d⁻¹]for the biofilm batch production can be calculated using the yearly productivity of one panel YB_{PAN}, the area of each panel A_{PAN} and the number of productive days per year (T_{BATCHPAN}, N_{CYCPAN}):

$$AP_{PAN} = \frac{YB_{PAN}}{A_{PAN}*T_{BATCHPAN}*N_{CYCPAN}}$$
(Eq. 5.26)

For a $T_{BATCH} = 10$ d, this results in an areal productivity of 0.9269 g m⁻² d⁻¹ for biofilms on PVDF and of 1.4014 g m⁻² d⁻¹ for biofilm on FG. Considering the differences in cultivation conditions and material, these values are remarkably close to Naumann et al. (2012) and indicate that the biofilm growth model is working as expected.

The semi-continuous systems could not be validated at this time, as no compatible data was available. However, considering that the underlying growth models and inputs are the same as in the batch cultivations, the results should still be reasonably accurate.

The same lack of data also prevents validation of the power consumption. The current estimates are based on the lights and procedures used at laboratory-scale – industrial

systems might use different set-ups. Further validation would be highly desirable for all production cases, especially against data obtained from industrial production systems.

Biomass productivity and system scale:

Base line process: Suspension batch

As shown earlier (Figure 5.4), for the baseline process the best yearly productivity of 240 kg yr⁻¹ is achieved in a cultivation cycle of 9 days with a growth period of 7 days. This coincides with the end of the first, faster growth phase shown in Figure 5.2. Under these conditions the growth always proceeds at its maximum rate, while the ratio between days of active growth ($T_{BATCHBAG}$) and unproductive days (T_{RESET}) is kept as large as possible.

Scenario 1: Suspension semi-continuous

The optimal number of bags for the semi-continuous suspension cultivation is 9 (Table 5.2), which equals a yearly biomass productivity of 270 kg yr⁻¹. This is higher than the target, as the number of bag systems must rounded up to the nearest integer – the productivity in 8 bags would 216 kg yr⁻¹. This productivity is achieved by cultivating the biomass until the end of the exponential phase ($T_{BATCH} = 7$ d), harvesting 138 l from each bag, which allows for regrowth in one day (T_{RG} of 1 d). In this manner, the system maximises the use of the faster growth rates at the end of the exponential phase, while keeping the biomass concentration and productivity as high as possible.

Table 5.2: Number of bags required for semi-continuous suspension cultivation The table shows the number of bags required to match the optimal productivity of the baseline process (240 kg yr⁻¹) for a given combination of T_{BATCH} and $T_{REGROWTH}$ in the semi-continuous system. For example, a T_{BATCH} of 12 d and a T_{RGSCB} of 8 days would require 21 bags.

Results for all valid combinations of T_{BATCH} and $T_{REGROWTH}$ are shown. Highlighted cells are results require less bags than the base scenario. The least number of bags required is 9, for T_{BATCH} = 7 d and T_{RGSCB} = 1 d.

		T _{BATCHSCB} [d]																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	1		41	31	23	17	13	9	37	36	34	34	32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17
	2			35	26	19	14	11	15	36	34	34	33	32	31	29	28	27	26	25	24	23	23	22	21	20	19	18
	3				30	22	16	12	14	19	35	35	34	33	31	30	29	28	27	26	25	24	23	22	21	20	19	18
	4					25	18	14	15	17	21	36	34	33	32	31	29	28	27	26	25	24	23	23	21	20	19	18
	5						20	15	16	17	18	23	35	34	32	31	30	29	28	27	26	25	24	23	22	21	20	19
	6							17	17	17	18	20	24	34	33	32	30	29	28	27	26	25	24	23	22	21	20	19
	7								18	18	19	20	22	25	32	32	31	30	29	28	26	26	25	24	23	22	21	20
	8									20	20	20	21	22	25	33	31	30	29	28	27	26	25	24	23	22	22	21
	9										21	21	21	22	23	26	31	31	29	29	27	26	26	24	24	23	22	21
	10											22	21	22	22	24	26	32	30	29	28	27	26	25	24	23	21	20
=	11												22	23	22	22	24	26	31	29	29	27	26	25	24	24	22	22
2	12													23	23	23	23	24	27	30	28	28	26	26	25	23	22	21
SCE	13														24	24	23	23	24	26	29	28	27	26	25	24	23	23
Le la	14															24	24	23	24	24	25	29	27	27	26	24	24	23
F	15																24	24	24	23	25	26	27	27	26	24	23	22
	16																	24	24	24	24	24	24	28	26	25	25	23
	17																		24	23	24	24	24	25	27	25	24	24
	18																			24	23	24	24	23	25	26	25	23
	19																				24	23	24	23	23	25	26	24
	20																					24	23	24	23	23	23	24
	21																						24	23	24	23	23	23
	22																							24	23	23	22	23
	23																								23	24	23	22
	24																									23	22	22
	25																										24	23
	26																											22

Scenario 2: Biofilm batch

The simulation of the biofilm batch production shows that the biofilm system requires considerably more panels than the suspension systems needs bags (Figure 5.10), in the order of 10 times more individual systems required. Fibreglass is more productive than PVDF, due to the higher inoculation density, despite the lower growth rates.

However, Figure 5.10 shows that both surface materials achieve the minimum number of panels at very short cultivation times. In practice, this suggests that the inoculum should be concentrated on the membranes and removed as quickly as possible, to maximise the number of cultivation cycles, which is essentially a filtration system rather than a cultivation system. When constraining calculations to those after the lag phase ($T_{CYC} \ge 5$), where growth actually occurs, cultivation on PVDF shows a local minimum of 216 Panels (Figure 5.10) for a cultivation time of twelve days ($T_{CYC} = 12$ d) corresponding to a growth phase of 10 days ($T_{BATCH} = 10$ d). This corresponds to the end of the exponential growth phase, as it has been observed for the suspension batch cultivation and as such this would be considered a biologically sensible result. On FG this local minimum is less apparent. The number of panels increases steadily with only a slight plateau, for 143 panels at T_{BATCH} = 10 d, T_{CYC} = 12 d.



Figure 5.10: Number of PMBR panels needed for microalgal biofilm batch cultivation. Cultivation on FG is overall more efficient. A 12-day cultivation cycle leads to a local minimum (PVDF) or slight plateau (FG). This corresponds to the duration of the exponential growth phase.

Scenario 3: Biofilm semi-continuous

Semi-continuous cultivation of biofilms (Table 5.3), results in some improvement for cultivation on PVDF, reducing the number of panels required to 135, for $T_{BATCH} = 10$ d and $T_{REGROWTH} = 1$, compared to 216 panels required for biofilm batch cultivation (for $T_{BATCH} = 10$ d). As in the semi-continuous suspension system, this represents cultivation until the end of the exponential phase and then harvesting daily to keep the growing biomass as high as possible. For a practical point of view, this harvesting schedule would require to remove only 6.4 % of the biomass each harvest – this could be

achieved by skimming an (automated) blade over the top of the biofilm, removing only the upper layers or by using a comb-like scraper (Boelee et al. 2013b).

Table 5.3: Number of panels required for semi-continuous biofilm cultivation

Results for all valid combinations of $T_{BATCHSCP}$ and T_{RGSCP} are shown, for PVDF (top) and FG (bottom). For PVDF the best combination requires 135 panels at $T_{BATCHSCBPVDF} = 10$ d and $T_{RGSCPPVDF} = 1$ d. The highlighted cells are combinations that require less panels than the 216 panels required for the biofilm batch cultivation at $T_{BATCH} - 10$ d. For FG, the best combination required 165 panels. None of the combinations required less panels than the biofilm batch cultivation.

														TBAT	CHPVD	_F [d]												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	1		3283	3283	295	260	229	202	178	156	135	579	573	567	561	555	549	544	538	533	528	522	517	510	499	489	479	469
	2			3283	534	276	244	215	190	165	143	223	575	570	563	558	551	547	540	536	529	525	519	513	502	492	482	472
	3				730	391	258	227	200	175	152	195	282	574	565	561	556	548	543	539	531	527	523	516	505	495	484	474
	4					496	329	241	212	186	161	190	235	326	570	563	556	554	547	540	533	532	525	519	508	498	487	477
	5						397	290	223	196	170	192	222	269	357	566	560	555	549	543	538	532	527	522	511	500	490	480
	6							336	260	207	179	197	219	251	295	380	563	558	553	549	537	533	529	525	514	503	493	482
	7								293	235	192	205	221	239	264	321	403	558	554	551	539	536	533	530	519	516	514	503
	8									266	213	212	225	238	255	295	337	415	560	548	546	544	533	531	520	518	517	506
_	9										234	233	232	240	259	276	300	359	425	555	543	542	541	530	530	519	518	507
P	10											256	248	247	257	277	295	330	369	440	547	548	536	537	525	514	504	493
, DF	11												266	266	258	266	293	310	345	382	451	547	536	537	526	528	517	519
PPP	12													288	280	272	290	302	330	354	388	454	540	542	531	520	509	499
GSG	13														294	296	288	295	306	341	364	397	460	537	541	530	519	523
۳	14															313	304	296	316	327	342	378	408	469	539	528	533	522
	15																317	321	312	319	344	358	379	423	465	528	517	506
	16																	328	319	324	330	338	350	397	424	463	539	527
	17																		333	324	337	344	353	383	402	427	462	533
	18																			347	337	345	351	360	390	407	430	463
	19																				354	345	353	359	367	398	414	436
	20																					363	353	364	369	376	387	401
	21																						365	356	367	370	375	382
	22																							377	367	358	361	399
	23																								367	389	379	383
	24																									389	379	370
	25																										406	395
	26																											395

														TBAT	CHSCPF	_{-G} [d]												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
	1		1274	1274	230	218	207	197	187	176	165	407	404	400	396	393	389	386	383	379	376	373	370	365	359	352	345	339
	2			1274	385	224	213	202	192	181	169	242	405	402	398	395	391	388	384	381	377	375	371	367	360	354	347	340
	3				496	304	218	207	196	185	173	217	280	404	399	396	393	388	386	383	378	376	374	369	362	355	349	342
	4					373	271	213	201	190	178	210	246	305	402	397	393	392	388	384	380	379	375	371	364	357	350	344
	5						318	251	205	195	182	208	234	269	320	400	396	392	389	386	382	379	376	373	366	359	352	345
	6							285	235	199	187	209	229	254	284	330	397	394	392	389	382	379	377	375	368	361	354	347
	7								262	223	194	207	221	235	253	299	341	394	392	390	383	381	380	378	371	370	368	361
	8									249	213	209	221	232	245	281	306	344	396	388	387	386	379	378	371	370	370	363
	9										232	227	223	231	246	258	273	317	347	393	385	385	385	378	378	371	371	364
	10											253	243	239	248	264	277	300	321	354	388	389	381	382	375	368	361	354
Ъ	11												252	249	240	247	274	286	309	327	358	388	381	382	375	377	369	371
9	12													274	264	254	269	278	298	311	328	357	383	385	378	371	364	357
BPF	13														269	268	258	264	271	304	316	331	359	382	385	378	370	374
gg	14															289	279	269	285	292	301	323	336	363	383	376	380	373
ΗĒ	15																289	289	279	285	302	311	320	344	358	376	369	362
	16																	287	277	279	283	287	293	332	342	355	383	376
	17																		288	279	295	299	305	324	332	342	353	380
	18																			304	295	298	302	307	327	334	342	352
	19																				308	298	303	306	310	330	336	344
	20																					312	303	308	311	315	320	325
	21																						303	294	300	302	303	306
	22																							309	300	292	293	328
	23																								300	323	314	316
	24																									323	314	306
	25																										333	324
	26																											324

In contrast, for FG, semi-continuous growth mode does not result in any improvement in the number of panels needed, with a minimum of 165, compared to the 143 for the biofilm batch at $T_{BATCH} = 10$. This can be explained by the high initial biomass and the low specific growth rates in this system – it is more efficient to re-inoculate the system than remove a small proportion and regrow it slowly.

Power consumption

Table 5.4 shows the power consumption of the different production processes, for each processing step per individual system and overall for the whole cultivation. The individual system with the lowest power consumption was the batch biofilm on PVDF at 2455 kWh yr⁻¹, the system with the highest power consumption was the semi-continuous suspension system, at 3056 kWh yr⁻¹. Overall, biofilm systems have slightly lower power consumption than suspension systems, explained by the lack of harvesting costs and the lower power consumption of recirculation compared to aeration.

Power consumption was dominated by light (76 – 89 %), followed by aeration and recirculation, for the respective systems – i.e. most power is consumed during the growth step of the process. Harvesting contributed very little to the power consumption (<2%) of the suspension systems.

The total yearly power consumption to match the baseline suspension batch process (i.e. 240 kg yr⁻¹) is largely a function of the number of individual systems needed as there is little difference between the consumption of the individual systems. Consequently, the semi-continuous suspension process shows the lowest yearly power consumption, 27,506 kWh yr⁻¹ and the lowest specific power consumption 102 kWh kg⁻¹, approximately halving power consumption compared to the baseline batch suspension process, with 52,156 kWh⁻¹ and 217 kWh kg⁻¹, respectively. In contrast, the biofilm systems require considerably more energy, due to the large number of panels required, resulting in 6 to 10 times higher power use.

Table 5.4. Comparison of power consumption.

The yearly power consumption for one bag or panel at best biomass productivity (as above). Power consumed is broken down processing step (boxes), both as power consumed (in KWh yr⁻¹) and as percentage of the total yearly power consumption. Power consumption for the total productions (i.e. to produce ~ 240 kg(DW) yr⁻¹) and specific power consumption as calculated

	Suspension						Biofiln	n batch		Biofilm semi-continuous					
	Ba	itch	Semi-co	ntinuous		P\	PVDF		G	P١	/DF	F	G		
	T _{BATCH} = 7		T _{BATCH} = 7, T _{RG} = 1			T _{BATCH} = 10		T _{BATCH} = 10		T _{BATCH} = 1	0, T _{RG} = 1	T _{BATCH} = 1	_{ATCH} = 10, T _{RG} = 1		
	KWh yr⁻¹	%	KWh yr⁻¹	%		KWh yr ⁻¹	%	KWh yr ⁻¹	%	KWh yr ⁻¹	%	KWh yr ⁻¹	%		
Inoculum	244	9.35	32	1.06	Inoculum	86	3.48	220	8.51	15	0.51	37	1.31		
Fill	0	0.00	0	0.01	Vacuum	0	0.00	0	0.01	0	0.00	0	0.00		
Light	1976	75.76	2506	81.98	Light	2117	86.23	2117	81.74	2519	88.89	2519	88.18		
Aeration	364	13.96	462	15.10	Recirculation	252	10.28	252	9.74	300	10.59	300	10.51		
Harvesting	24	0.93	56	1.85											
Total per system	2608	KWh yr ⁻¹	3056	KWh yr ⁻¹	Total per system	2455	KWh yr ⁻¹	2590	KWh yr ⁻¹	2834	KWh yr ⁻¹	2857	KWh yr ⁻¹		
# Bags	20		9		# Panels	216		143		135		165			
Total production	52,156	KWh yr ⁻¹	27,506	KWh yr ⁻¹	Total production	530,215	KWh yr ⁻¹	370,330	KWh yr ⁻¹	382,560	KWh yr ⁻¹	471,361	KWh yr ⁻¹		
Specific power	217	KWh kg⁻¹	102	KWh kg ⁻¹	Specific power	2209	KWh kg⁻¹	1543	KWh kg⁻¹	1594	KWh kg⁻¹	1964	KWh kg ⁻¹		

5.6 Conclusions

The simulation successfully compared suspension and biofilm cultivation using inputs obtained under comparable conditions and extrapolated potential production at small and medium sized enterprise scale. The model produced realistic productivity results and was used to optimise process outcomes by testing different alternatives.

Accuracy of the simulation is currently limited by the direct transition from laboratoryscale to production-scale and by the number of assumptions made about the overall cultivation process, for example T_{RESET} . This could be improved by using inputs obtained from larger scale, ideally commercial production systems. Other power costs, such as air-conditioning (FinFishEnterprise 2015), other OPEX (eg. labour, consumables) and CAPEX should also be included and can easily be integrated into the current model. Further process alternatives, such a different concentration systems or lighting options can also be investigated.

A sensitivity analysis should be included to assess the relative effect of changes in different cultivation parameters on the overall outcome of the cultivation. Light quantity and quality would be an obvious choice, due to the large contribution to the power costs of the process and due to its inherent importance for microalgal photosynthesis (Garcia-Camacho et al. 2012; Schultze et al. 2015) in general, and on the growth rate of TISO in particular (Marchetti et al. 2012). This would be especially worthwhile once OPEX and CAPEX have been sufficiently included to allow a complete techno-economic analysis.

Also of interest would be changes in the specific growth rate μ for regrowth. In the current model, the μ for regrowth is identical to that of the original cultivation, justified by the limitations in the available data. However, there are indications that regrowth of a harvested culture is faster than original growth, as the cells are already adapted to their environment. This has been found in laboratory-scale suspension cultivation of TISO (Huerlimann et al. 2010) and in laboratory-scale (Johnson and Wen 2010) and pilot-scale (Boelee et al. 2013b) cultivation of mixed species biofilms. Faster regrowth could increase the productivity of semi-continuous approaches and should be further investigated.

With regard to the specific results for this analysis, biofilm cultivation shows lower productivity per system than the bag system. As shown in table 5.4, taking into account the 2 m² of cultivation area per panel, in batch cultivation 21.6 m² of biofilm on PVDF would be needed to match the

Table 5.5 Biofilm area to match bag production

For production of 240 kg(DW) yr ⁻¹ :		
Suspension batch	20 bags	
		m ² bag ⁻¹
Biofilm batch (PVDF)	216 panels	21.6
Biofilm batch (FG)	143 panels	14.3
Suspension semi-continuous	9 bags	
		m ² bag ⁻¹
Biofilm semi-continuous (PVDF)	135 panels	30.0
Biofilm semi-continuous (FG)	165 panels	36.7

productivity of a 500 l bag and 30 m² of area per bag in semi-continuous cultivation. The numbers are similar for FG, with 14.3 m² and 36.7 m², respectively.

While this does represent an increase in infrastructure compared to the bag cultivation system, it still shows that the biofilm systems can operate at a comparable scale. As the research into applied biofilm cultivation progresses, there is considerable potential for improvement. System design and process understanding are constantly improving and some of the techniques for achieving very high productivities in other systems (eg. Liu et al. (2013), Gross et al. (2015b), Schultze et al. (2015)) might be transferable to TISO (Marchetti et al. 2012).

Immediate potential seems to lie in the adaptation of a semi-continuous cultivation strategy, even without fundamentally changing the underlying infrastructure. The improvement shown in the current results should be further investigated and verified in more comprehensive studies. Changes should be evaluated for their effect on the production process as it is actually implemented and will need to be suitable to the available infrastructure. But process optimisation in this regards appears worthwhile, even if only a portion of the benefit shown in these results can be transferred.

Another refinement of the cultivation would be to include biomass quality parameters, such as protein content or fatty acid composition of the biomass, as this has been shown to vary over the age of the culture (Huerlimann et al. 2010). This would allow to optimise production for product quality, rather than biomass productivity. However, such investigations would need to be coordinated with the scale-up efforts mentioned above to include the appropriate cultivation conditions.

The underlying mathematical models could be refined to include more factors – for example nutrient saturation kinetics and light penetration (Quinn et al. 2011; Costache et al. 2013) or the interaction of different cultivation conditions (Garcia-Camacho et al. 2012). There are many refined models for biofilm cultivation that are currently being postulated, including Wolf et al. (2007), Liao et al. (2012), Cumsille et al. (2014) and Munoz Sierra et al. (2014). The challenge will be to choose wisely to achieve the necessary degree of accuracy, while maintaining the ability to determine the necessary input parameters from relevant systems – an empirical approach, where simpler models are fitted might be more efficient. Sensitivity analyses would be required to quantify the most important parameters affecting the overall outcome, CAPEX and OPEX.

In conclusion, this work shows the feasibility of comparing biofilm and suspension cultivation with mathematical models. The results are sound and biofilm cultivation can be achieved at comparable scale to suspension systems. The results should be further refined and verified in the appropriate industrial context. With improved data, this work could be expanded into a complete techno-economic analysis to guide future process development.

5.7 Acknowledgements

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Chapter 6: General discussion

6.1 Summary

The research presented in this thesis aimed to improve the current microalgal bioprocess technology, dominated by suspension systems, for the production of biomass in industrial application. Specifically, it explored the potential of using microalgal biofilm cultivation to improve cultivation outcomes and reduce harvesting/dewatering effort. This was achieved by assessing the current state of the field in a literature review and categorisation of biofilm cultivation strategies, by developing a prototype cultivation system and testing it under tropical conditions, by developing a laboratory assay for more detailed investigations of perfused biofilms and by simulating production processes for biofilm and suspension cultivation in an aquaculture context.

Below, the conclusions presented in each chapter are briefly revisited and considered in the larger context of this work. The overall knowledge gained across the whole thesis is discussed and an outlook for future research and application of microalgal biofilm processes is provided.

6.2 Main conclusions and outcomes

The published review in chapter 2 (Berner et al. 2014) provided the first comprehensive overview of microalgal biofilm production systems and categorised these systems into three different types based on the exposure of the biomass to the cultivation medium: constantly submerged systems, intermittently submerged systems and perfused systems. The review highlighted the prevalent and rapid innovation in the field. It also identified critical knowledge gaps and the need for increased standardisation between systems.

Most of the systems described in this review have emerged during the course of this PhD research, which started in early 2011. At that time, the published microalgal bio-film production systems consisted of variations of algal turf scrubbers (Adey et al. 1993; Adey et al. 2011) and a number of laboratory experiments not intended for large-scale production, e.g. (Shi et al. 2007; Zippel et al. 2007; Johnson and Wen 2010).

The number of relevant publications have greatly increased since then, showing an increased interest in the potential of microalgal biofilm production, see Gross et al. (2015a) for another recent review.

Since the publication of the review in 2014, a number of publications of particular interest to this thesis have been published. The twin layer photobioreactor has been further developed and has been used in two further studies. Shi et al. (2014) investigated the use of the system for wastewater treatment and reported N & P removal rates of 70 to 99 % while growing *Halochlorella rubescens* with an areal growth rate of 6.3 g m⁻² d⁻¹. The same species was used by Schultze et al. (2015) in the same system, but under high light and high CO₂ conditions – concepts introduced by Liu et al. (2013), a sign that some standardisation is occurring across the field, which was one of the key conclusions emphasised in Chapter 2. In this study, productivities of up to 31.2 g m⁻² surface area were achieved and up to 52 g m⁻² d⁻¹ per area of footprint, for closely spaced panels. These very high growth rates at pilot-scale demonstrate the potential of this reactor design.

Meanwhile the multiplate system by Liu et al. (2013) has been applied in several studies for the cultivation of high-value bioproducts, especially pigments. This includes work with *Pseudochlorococcum*, (Ji et al. 2014a), *Acutodesmus obliquus* (Ji et al. 2014b), *Botryococcus braunii* (Cheng et al. 2014) and *Haematococcus pluvalis*. (Zhang et al. 2014).

Another development occurred with the revolving algal biofilm system's (RAB) unique conveyor belt type system (Gross et al. 2013) that was at laboratory-scale when it was reviewed for Chapter 2. This system has been scaled up to pilot-scale (Gross and Wen 2014) and has been tested over the course of a year, with an average biomass productivity of 5.8 g m⁻² d⁻¹ per surface area and 18.9 g m⁻² d⁻¹ per areal footprint. This system offers an interesting alternative for the enhancement of existing ponds, similar in application, if not design, to the systems used by Christenson and Sims (2012) and Orandi et al. (2012). Further comparisons of these systems would be desirable – a simulation as described in chapter 5, could assess if there is a substantial benefit gained from the increased complexity of the RAB, for example. However, it would have to account for the number of compounding factors, which limit meaningful

comparisons (as noted in Chapter 2) and there might be impetus for the development of more of standardised tests, as described in chapter 4.

Chapter 3 described the development of prototype perfused biofilm reactors. The feasibility of this approach was demonstrated and evaluation of the experimental problems highlighted challenges associated with photosynthetic biofilm cultivation in the tropics. The brevity of the final publication belies the amount of work that went into system design and prototype development, as the substantial uncertainty and the sparsity of design examples during the design and construction period required several iteration of prototype modification during development. The experimental work was ultimately constrained by the available infrastructure and by the absence of literature associated with microalgal biofilm cultivation in the tropics. This has led to results that are not entirely satisfying, with regards to system productivity and stability in extreme temperatures but has also highlighted the need for further investigations and the importance of research under appropriate environmental conditions.

The RPM-PBR design, described in Chapter 3, offers interesting possibilities in view of the use of light dilution which resulted in very high areal productivities (Liu et al. 2013; Schultze et al. 2015). These systems achieved high productivities with light dilution factors (R_L) of 4 to 20 (cultivated growth surface area A_C per illuminated (footprint) area A_L) by arranging several closely spaced vertical cultivation panels parallel to each other. Light dilution could also come into effect in the RPM-PBR, although in a slightly different manner (Figure 6.1), as the vertical panels aren't parallel to each other, but rather radially arranged around a central shaft.



Figure 6.1: Illustration of the light dilution factor (R_L) in a 6 panel RPM-PBR. *Left*: Impression of a 6 panel RPM-PBR. *Right*: Top view of the same system, illustrating the relationship between the illuminated area (A_L) and cultivated area (A_C), dependent on the radius r and the angle α of the panels.

In the PRM-PBR the illuminated area (A_L) and the cultivated area (A_C) can be calculated from the number of radially arranged panels (n), as per Eq. 6.1 – 6.5, where α is the angle between two panels and h is the height of the system, which is mathematically eliminated in the light dilution factor (R_L).

$R_L = \frac{A_C}{A_L}$	Eq. 6.1
$A_L = 2 * \pi * \sin\frac{\alpha}{2} * h$	Eq. 6.2
$A_{\mathcal{C}} = 2 * r * h$	Eq. 6.3
$\alpha = \frac{360^{\circ}}{n}$	Eq. 6.4
$R_L = \frac{1}{\sin\frac{\alpha}{2}} = \frac{1}{\sin(\frac{360^\circ}{2n})}$	Eq. 6.5

A larger number of radial panels results in larger light dilution, due to smaller α (Table 6.1). While R_L is smaller than in parallel plate systems for anything other than a very large number of panels, there is also potential improvement due to intermittent illumination.

Number of panels	α	RL
4	90°	1.41
6	60°	2
8	45°	2.61
10	36°	3.24
20	18°	6.39

Table 6.1: R_L for different number of panels in an RPM-PBR

The rotation of the system leads to intermittent illumination, i.e. each panel would only be exposed to a burst of light for a small period of its total rotation, dependent on the number of panels. Intermittent illumination has been shown to have a positive effect on photosynthetic efficiency and growth in microalgae (Grobbelaar 2010), including an increase in lipid content and productivity in TISO (Yoshioka et al. 2012). In view of the high light intensities observed during the cultivation of the RPM-PBR, this indicates that rotating the system could have the potential for high biomass growth in tropical regions. Of course, there are numerous additional factors to consider that are beyond this brief reflection – such as the environmental conditions, rate of rotation and frequency of light, angles of illumination, microalgal species and nutrition, just to name a few.

Also of interest would be conceptual combinations between the RPM-PBR and other rotating biofilm systems, such as the Rotating Algal Biofilm reactor (RABR) (Christenson and Sims 2012) and the photo-rotating biological compactor (PRBC) (Orandi et al. 2012). Each of these systems employs a different arrangement of the cultivation surface: Radially in the RPM-PBR, along the circumference in the RABR and

parallel plates in the in the PRBC (which could also include an element of light dilution). While the systems have little in common otherwise, so far, there could be interesting comparisons of the effect of system geometry in otherwise identical circumstances.

The development of the Petri-dish assay in chapter 4 was motivated by the lack of standardisation observed in chapter 2 and by the experience gained during system development in chapter 3, where fundamental questions, such as the choice of surface materials or inoculation densities, could only be addressed after building a prototype. The complexity and variability in system design at pilot and full scale, and the range of contributing factors affecting the process outcomes, highlight the need for a standardised approach where multiple factors can be assessed rapidly.

The Petri-dish assay has been established as a laboratory procedure and has been successfully used to investigate the fundamental factors, such as light levels, surface material and species, and the interaction thereof. It has also provided the TISO data that provides the biological growth input for the mathematical models in Chapter 5, showing possibility for the wider applications of these assays. Chapter 4 is currently in preparation for publication, which will hopefully lead to wider use of this approach and increased use in parallel with larger systems to help relate laboratory scale results into a larger, more applied context.

In Chapter 5, a productivity and power model was used to compare microalgal production in suspension and biofilm systems at the scale of a small to medium enterprise. This combines the fundamental layout of a system described in chapter 2, (Naumann et al. 2012), with my personal experience gained during the construction of the prototype biofilm reactors in chapter 3 and the experimental data produced with the Petri-dish assay developed in chapter 4.

The simulation shows that adaptation of a semi-continuous cultivation mode could lead to reduced power costs, but also that biofilms will still need some improvement to be competitive. However, in view of the rapid development seen across the field and some of the new production systems discussed in Chapter 2 these improvements might not be far off. With the appropriate, industrially relevant background data, the

work could be expanded into a techno-economic analysis to be the first of its kind focussing specifically on the cultivation of microalgal biofilms in aquaculture for the production of much needed live microalgal paste (Tredici et al. 2009; Hemaiswarya et al. 2010).

In view of the increasingly larger number of cultivation systems and species being investigated for microalgal biofilm production, mathematical simulations are increasingly important, as they enable comparative assessment of system and process alternatives, allow techno-economic considerations to be quantified and guide future research and development.

6.2 Outlook

As it was five years ago, microalgal bioprocess technology remains a field of great interest and with many opportunities for future applications. As highlighted throughout the thesis, a number of areas were identified that are of special interest:

Process integration, modelling and standardisation

The knowledge and innovation demonstrated at laboratory- and pilot-scale needs to be integrated into the larger industrial context and proven in successful applications. This can only happen in cooperation with interested industries in the context of appropriate applied research projects. These projects will by necessity be interdisciplinary, requiring an understanding of biology, underpinned by process engineering and fundamental economic aspects. Techno-economic analyses based on accurate productivity and power models will be valuable tools to determine promising production pathways and likely outcomes. However, these tools are only as good as the data used for their calculations and increased standardisation of analytical methods and processes would greatly contribute to the reliability of available data.

Clever system engineering

In addition to the applied research above, there is also still considerable room for innovation, by finding clever combinations of fundamental biological processes and system design. The understanding of biofilm growth and biology has increased over the last decade and several functional models are now available to provide mathematical descriptions of the internal processes in biofilms. Combining these

models with process models from system engineering has the potential to highlight optimal conditions that are not *a priori* apparent, but might provide decisive advantages with regards to process outcomes and lead to optimised system designs.

Key challenge: Harvesting

How to effectively harvest biofilms has received very little attention in the literature. Most research groups seem to simply adopt a method that suits their needs and capabilities related to system in use at the time. Scraping the biofilms of the surface is a very common approach, usually with a handheld tool or scraper (Johnson and Wen 2010; Naumann et al. 2012; Boelee et al. 2013b) although an integrated harvesting mechanism has been proposed by Christenson and Sims (2012). Another option is to vacuum the biofilm of the surface, an approach that lends itself to large applications as commercially available wet vacuums can be used (Craggs et al. 1996b). Already used in an automated commercial pilot system is the option to rinse the algae off the surface with high-powered water jets (Bioprocess Algae 2012), although this does dilute the high cell concentration of the biofilm somewhat, negating one of the key benefits of biofilm cultivation. Furthermore, the water use needs to be considered in the overall water requirements for the process.

For efficient larger scale production, the harvesting methods will need to be integrated into the production process, so that the harvested biomass can be passed from cultivation to downstream processing with minimal weight loss and quality degradation. In production facilities with low labour costs, manual harvesting may be an economically attractive option, but in industrialised countries, a high degree of automation will be required. How this will affect overall process costs will need to be further investigated as system designs are narrowed down to specific species, applications and locations.

The biological effects of harvesting should also be further investigated, i.e. how does a harvesting regime affect the cells in the biofilms and the regrowth of the biofilm. Initial studies by Boelee et al. (2013b) have shown that harvesting the top layer of thick mixed wastewater biofilms is preferable to harvesting the whole biofilm completely or to harvesting thin biofilms. However, this is the only study of its kind to date and

further investigations are required to ascertain how harvesting affects single species biofilms and how harvesting methods and frequency affect biomass composition and regrowth characteristics and productivities.

Key challenge: Surface material

The surface material used for biofilm cultivation is another key technical aspect that deserves further attention, both for biological and system engineering aspects. With regards to biology, surface characteristics play a very important role early in biofilm development, as the cells settle onto the surface or the early conditioning films establish (Costerton et al. 1995). The interaction between cells and surface materials are critical in determining adhesion strength of the biofilm which influences sloughing. Some early, mostly empirical screening has been conducted by several groups. (Cao et al. 2009; Johnson and Wen 2010; Christenson and Sims 2011; Ozkan et al. 2012; Cui et al. 2013), but, as with system designs, there is a distinct lack of a standardised approach that could lead to a general explanation of surface interactions. While there is considerable ongoing research into the fundamentals of cell/surface interactions, it is mostly focussed on biofilm avoidance or removal, for example in medical or nautical applications (Schultz et al. 2011), rather than biofilm cultivation. However, the fundamental mechanisms identified could potentially cross-inform decision making for the purposeful cultivation of biofilms.

Practical aspects of surface materials also need to be considered for large-scale cultivation, namely cost of the material, durability, life-time and recycling or removal. Current small-scale systems are mostly built using materials that are easily obtainable and easily modified by researchers in biology laboratories, but might be not suitable for large-scale use due for a variety of reasons. For example, cultivation in perfused membrane reactors in this work and others (Naumann et al. 2012; Liu et al. 2013) has been conducted on commercially available membrane filters and on newspaper, both of which would pose additional challenges at larger scale. Membrane filter materials would be very expensive for systems with potentially hundreds of square meters of cultivation area and would consequently need to be reused several times to justify the investment. In contrast, while newspaper is cheap and easily available in any size, it lacks mechanical ruggedness and can easily tear while being installed in the system or

during harvesting. There are, however, thousands of different kinds of membrane filers and paper formulations, providing undoubtedly much potential for selection of membrane material with optimal properties regarding cost and stability, whilst simultaneously also satisfying biological criteria as mentioned above.

Understanding fundamental mass transfers

The mass transfers between the different compartments of a biofilm reactor are complex, and especially so for perfused membrane photo-biofilm reactors (Figure 6.2). Identifying and quantifying the flow of diverse chemicals that move between biofilm, base layer and environment would be critical to develop an in-depth understanding of fundamental processes of such systems. Flow complexity is a result of impacts of fundamental substrate attributes such as the porosity of the membrane or the diffusivity of the biofilm, but these factors are also not consistent spatially over time (e.g. membrane degradation or biofilm age) and space (biofilms at the top or bottom of the system are different, as seen in Chapter 3).

Future research reactors should be designed from the beginning with this in mind and should provide the analytical tools to measure the necessary concentrations and environmental conditions with the adequate temporal and spatial resolution, an example of such a system can be found in Murphy and Berberoglu (2014). Together with suitable mathematical models, such integrated measurements can also be used for in-line and on-line process monitoring and control.



Figure 6.2: Overview of mass transfers in a perfused photo-biofilm reactor.

The biofilm exchanges molecules both with the environment and into the liquid conducting base layer, across the membrane. Furthermore, there is constant recirculation of molecules between the different zones in the biofilm.

Preferred applications for biofilms

The main advantages of biofilm cultivation systems compared to suspension systems are the adherence of cells to a surface, the higher cell concentration and the lower water volumes potentially without open water surfaces, e.g. in perfused systems. Main disadvantages are the overall higher complexity of the system design and the need for environmental control.

As such, biofilms are well suited for use in urban areas and indoor cultivation, where space is a premium. Vertical panels allow for high ratios of cultivation surface per areal footprint and could be installed on existing vertical walls. The lower water requirements keep pumping costs low, compared to a similarly sized flat panel reactor. As such, biofilm panels could contribute to green cities and could also provide the means for localised recycling of CO_2 and waste waters in urban areas. A somewhat extreme example of this is the use of biofilms in life support systems in space crafts, as proposed in Murphy et al. (2013) and Verrecchia et al. (2015).

Vertical panels can also be used in very high-light environments, e.g. near the equator in the tropics, making use of light dilution (Liu et al. 2013) to avoid light inhibition of the cells. As these environments are also often experiencing high temperatures they also experience high evaporation rates from open water surfaces. The precise control over water flows that is possible in perfused systems would avoid this problem, while actively using the evaporation to cool the cells. Ideally the water is then reclaimed and recycled. As the tropics are a global region with increasingly fast socio-economic development and are predicted to accommodate more than half of the global population by 2050 (State of the tropics 2014). High light intensities and year-round growth periods provide ideal conditions for the cultivation of photosynthetic biomass. For this reason, there is great opportunity in testing – and adapting systems that have been researched under temperate conditions.

The high cell concentrations in biofilm systems effectively eliminate the first step of downstream processing, i.e. cell capture and concentration. This reduces the mechanical stress that the cells experience after cultivation and reduces the time until the cells can be conserved with other methods, for example flash-freezing. As such, this approach to cultivation well suited for the production of sensitive biomolecules that need to be isolated and conserved as fast as possible, in medical or nutraceutical applications.

Aquaculture provides an ideal environment for applied research into microalgae bioprocess technology. The industry is globally expanding while also increasingly transforming from low cultivation intensity to better controlled, high intensity production incorporating many principles of bioprocess technology. Microalgae are already familiar to the industry and have existing applications that can serve as a baseline for future improvements. Microalgal feeds can be produced at the scale of several hundreds to few thousands of kg per year and can still generate substantial revenue. Specifically microalgal biofilms remain of interest for the production of microalgal concentrates and for use with grazers that naturally feed on biofilms.

Microalgal biofilms and wastewater treatment have a long standing association and many systems have been developed in this context. As the overall industry of

microalgal cultivation matures, this also creates new markets and opportunities to integrate remediation and bio-product development.

6.3 Overall conclusion

This thesis has provided the first comprehensive review of microalgal biofilm production systems, which led to the development of perfused bioreactor prototypes and the identification of new challenges when cultivating microalgae in tropical conditions. A laboratory-scale assay for perfused biofilms was developed and established as a laboratory procedure. Data produced and experience gained was integrated into the simulation of productivity and power costs of the cultivation of aquaculture concentrates. Overall, this work successfully applied perfused biofilm cultivation principles to improve microalgae production and help unlock the future potential of applied microalgal biotechnology.

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Appendix 3.1: Provisional patent application

TITLE

BIOREACTOR AND METHOD OF USE

FIELD OF THE INVENTION

THIS INVENTION described herein relates generally to a bioreactor for cultivating microorganisms. In particular, the invention relates to a bioreactor, method and system for cultivating photosynthetic microorganisms.

BACKGROUND TO THE INVENTION

Culture systems for microorganisms are generally classified according to their engineering and hydraulic characteristics in open systems, examples of which include ponds, deep channel and shallow circulating units and closed or fully hydraulic systems commonly called bioreactors or photo-bioreactors (if cultured organisms are capable of using light as an energy source). Most microorganisms and in particular, photosynthetic microorganisms, are capable of growing both in suspension cultures and as a biofilm attached to a surface (examples of which include benthic microorganisms such as algae, microalgae and cyanobacteria).

When algae and other benthic microorganisms are grown as a biofilm attached to a cultivating surface, the biomass is naturally concentrated (containing less water) and more easily harvested, leading to more direct removal of the algal biomass and reduced processing. The cultivation of algae is currently being considered for a number of different applications, including: removal of CO_2 or other gases from industrial flue gases by algae bio-fixation; the reduction of

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Green House Gas (GHG) emissions from a company or process while producing biodiesel; wastewater treatment through the removal of unwanted compounds; the production of biomass for processing into ethanol and methane, the production of hydrogen gas, livestock feed, use as organic fertilizer due to its high N:P ratio,

5 energy cogeneration (electricity and heat); and the extraction of compounds including a large range of fine chemicals and bulk products, such as fats, polyunsaturated fatty acids, oil, natural dyes, sugars, pigments, antioxidants and high-value bioactive compounds. Other cultivatable microorganisms include but are not limited to methanotrophic bacteria for the remediation of methane, a GHG
10 with a warming potential 23 times that of CO₂ over a 100 year period and saprophytic fungi (for the treatment of problematic waste waters with a high load of complex and often chemically inert organic materials). The biomass of these organisms can be utilised as described above for algae and microalgae.

Because of this variety of high-value biological derivatives, with many possible commercial applications, microorganisms, in particular phototrophic microorganisms, could potentially revolutionise a large number of biotechnology areas including biofuels, cosmetics, pharmaceuticals, nutrition and food additives, aquaculture, and pollution prevention.

SUMMARY OF THE INVENTION

20 With such an important commodity, an improved bioreactor, method and system for cultivating microorganisms, in particular photosynthetic microorganisms, including algae and microalgae, is required.

Accordingly, it is an aim of the present invention to provide an improved bioreactor, method and/or system for cultivating microorganisms, in particular photosynthetic microorganisms.

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The present invention has arisen, after the inventors discovered a novel bioreactor system for the cultivation of microorganisms. The system provides a bioreactor and method for generating high density cultivated biomass with an increased productivity and reduced harvesting time when compared to existing technologies.

The present invention is broadly directed to a bioreactor and/or a method and system for cultivating microorganisms, preferably photosynthetic microorganisms.

In one aspect, although not necessarily the broadest aspect, there is provided a bioreactor comprising:

at least one liquid permeable layer comprising a first surface and a second surface;

wherein microorganisms are cultivatable on the first surface of the liquid permeable layer and the second surface is connectable in fluid communication with a fluid source; and

wherein the liquid permeable layer is rotatable.

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In a second aspect of the invention, there is provided a method for cultivating microorganisms, the method comprising:

rotating at least one liquid permeable layer comprising a first surface and a
 second surface, wherein the second surface is in fluid communication with a fluid source while cultivating the microorganisms on the first surface.

In a third aspect of the invention, there is provided a system for cultivating microorganisms, the system comprising:

at least one liquid permeable layer comprising a first surface comprising
 microorganisms and a second surface connected in fluid communication with a fluid source; and

a rotation member for rotating the liquid permeable layer, to facilitate microorganism growth.

The liquid permeable layer is rotated or rotatable to provide the microorganisms being cultured with sufficient conditions for maximum growth, such as for example, light and CO₂.

In one embodiment, the bioreactor further comprises at least one rotation member.

Suitably, the rotation of the at least one liquid permeable layer is facilitated by said rotation member.

The rotation member may be manually operated or may be automated. For example, the rotation member may be rotated or rotatable by a handle or automated by a motor or wind, solar and/or water powered apparatus, or a combination of the above.

In one embodiment, the at least one liquid permeable layer may be rotated or rotatable by a handle.

In another embodiment, the at least one liquid permeable layer may be rotated or rotatable by a motor.

In one embodiment, the at least one liquid permeable layer may be rotated or rotatable by wind, solar and/or water powered apparatus.

In one embodiment, the at least one liquid permeable layer is rotated or rotatable continuously.

In another embodiment, the at least one liquid permeable layer is rotated or rotatable intermittently.

In one embodiment, the rotation member rotates a platform comprising said at least one liquid permeable layer. Preferably said at least one liquid permeable layer is rotated or rotatable about an axis. More preferably said at least one liquid permeable layer is rotated or rotatable about a central axis of the liquid permeable layer or the bioreactor.

Suitably, rotation provides light exposure to a substantial portion of the first surface of the at least one liquid permeable layer.

In one embodiment, the bioreactor comprises a single liquid permeable 25 layer.

In a particular embodiment, the single liquid permeable layer may be cultivated with microorganisms.

Preferably, the single liquid permeable layer may be cultivated with photosynthetic microorganisms.

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In another embodiment, the bioreactor comprises a plurality of liquid permeable layers. In a particular embodiment, the plurality of liquid permeable layers may be cultivated with microorganisms.

Preferably, the plurality of liquid permeable layers may be cultivated with photosynthetic microorganisms. 5

Suitably, the liquid permeable layers may be provided in a double layer, between which a fluid source is located. The liquid permeable layers may comprise a first surface upon which microorganisms may be cultured to form a biofilm and a second surface connectable in fluid communication with the fluid source. In one embodiment, the same fluid source supplies fluid and nutrients to both liquid permeable layers. In one embodiment, a separate fluid source for each liquid permeable layer may be provided.

In one embodiment, the liquid permeable layer is configured as a sheet. The sheet may be of any size or shape, according to the culturing requirements of 15 the microorganisms. Shapes contemplated by the invention, include without limitation, cylindrical, octahedral, tetrahedral etc.

In one embodiment the liquid permeable layer is substantially cylindrical.

The liquid permeable layer acts like a membrane allowing fluid to pass from the second surface of the liquid permeable layer through to the first surface 20 of the liquid permeable layer, upon which microorganisms are capable of being cultured.

Preferably, the liquid permeable layer does not allow the passage of microorganisms from the first surface of the liquid permeable layer to the second surface of the liquid permeable layer, thereby reducing the risk of contamination. The liquid permeable layer may be formed from porous and/or perforated material.

Preferably the liquid permeable layer is formed from porous and/or perforated material that allows the selective passing of fluid. Such materials may include but are not limited to any woven, knitted, pleated, printed (e.g., by a 3D printer), felted or otherwise cross-linked synthetic or natural material (e.g., cotton,

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wool, silk, tree fibres, celluloses, including differing grades of paper (e.g., - acetate, -sulfonate, nitro-), chitins (e.g., sponges)) or synthetic polymers (e.g., polyesters, artificial sponges, polyacrylates, polyesters, polyamines, polysulfone, polyamides) or specifically nanotechnologically engineered 3-D nanofibres of either natural or synthetic origin, with or without surface modifications, (e.g., bonded enzymes, adhesive proteins etc.); polystyrene, silicon, nitrocellulose, cellulose acetate, glass fibre, polycarbonate, polyethylene, ceramics, glass, or metallic surfaces. Suitably, these materials may be used alone or in combination and may be modified to allow for the cultivation of a selected microorganism.

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10 In one embodiment, the fluid source may comprise any one or more of the following, without limitation: water; nutrients; water and nutrients; growth media for microorganism cultivation; waste waters and/or secondary treated sewage.

In one embodiment, the waste water and/or secondary treated sewage may be treated and/or filtered by the microorganisms.

- In a particular embodiment, the fluid source is provided in the form of a fluid conducting layer. Preferably, the fluid conducting layer is in fluid communication with the second surface of the liquid permeable layer. Preferably the fluid conducting layer is configured to provide fluid to a substantial portion of the liquid permeable layer.
- Suitably, the fluid conducting layer comprises a fluid conducting material, examples of which include but are not limited to: fabric; foam; glass fibres; synthetic polymers (e.g., polyesters, polyacrylate, polyamine, polyamide, artificial sponge etc); natural fibres (e.g., cotton, wool, sponge, hemp, tree fibre etc); 3D materials (e.g., meshed nano-fibres, spun or printed 3D matrixes) or a combination thereof. Preferably, the fluid conducting material comprises fabric. The fabric may be woven, knitted, a felt, mesh, cross-linked, or a combination thereof. More preferably, the fluid conducting material comprises capillary matting. The fluid conducting material provides at least fluid and/or nutrients and/or support to the growing microorganisms.
- 30 In one embodiment, the bioreactor further comprises a fluid reservoir.

Suitably, the fluid reservoir supplies the fluid source with fluid and optionally nutrients. Suitably, the fluid source may be transported from the fluid reservoir to the second surface of the liquid permeable layer.

In one embodiment the bioreactor further comprises a pump. Preferably, fluid may be pumped from the fluid reservoir to the second surface of the liquid permeable layer. More preferably, fluid may be pumped from the fluid reservoir to the fluid conducting layer. Alternate examples of fluid transport, include without limitation, capillary action, gravity, rotational forces, etc.

In one embodiment, the bioreactor further comprises a light source.

Preferably, the light source provides light to a substantial portion of the first surface of the liquid permeable layer.

In one embodiment, the light source provides continuous light.

In another embodiment the light source provides intermittent light. The light source may provide a combination of both continuous and intermittent light. 15 The light requirements may depend on the requirements of the microorganisms. The light source may be provided by natural light (e.g., sunlight) or may be provided by an artificial source such as a lamp.

In a further embodiment, the bioreactor is contained within a housing. Preferably, the housing allows light to penetrate through to the culturing microorganisms. More preferably, the housing is transparent.

In one embodiment, the bioreactor further comprises a gas source for the supply of CO_2 or other gases to the liquid permeable layer (e.g., methane). The gas requirements may depend on the requirements of the microorganisms for facilitating growth.

The present invention is applicable to all microorganisms. Preferably, the microorganisms are photosynthetic microorganisms. More preferably, the microorganisms are algae, microalgae or cyanobacteria.

Suitably, the microorganisms are capable of forming a biofilm, examples of which include, without limitation: photosynthetic microorganisms (e.g., algae,

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microalgae and blue algae); methanotrophic microorganisms; yeast; benthic microorganisms and combinations thereof.

More suitably, the photosynthetic microorganisms include cyanobacteria, the Rhodophyta (red algae), the Chlorophyta (green 5 algae). Dinophyta, Chrysophyta (golden-brown algae). Bacillariophyta Prymnesiophyta (haptophyta), (diatoms). Xanthophyta, Eustigatophya, Rhaphidophyta and Phaeophyta (brown algae). Non-limiting examples of photosynthetic microorganisms that form biofilms include: Scenedesmus obliquus (Chlorophyta), Isocrysis (haptophyta), Nannochloropsis (Eustigatophya), glabana sp.. 10 Tetraselmis Suecica (Chlorophyta), Phaeodactylum tricornutum (Bacillariophyta), Botryococcus braunii (Chlorophyta) and Spirulina (Cyanobacteria).

According to the invention, after cultivation, the microorganisms may be harvested from the liquid permeable layer. Harvesting may occur through loosening the microorganisms from the liquid permeable layer with a variety of means, examples of which include, although are not limited to physical scraping, fluid washing, chemical treatment, agitation, mechanical manipulation and/or drying. Harvesting may be undertaken with the liquid permeable layer in place, or after removal of the liquid permeable layer.

In one embodiment, the microorganisms are harvested from the liquid permeable layer when the desired level of biomass is cultivated.

In a further aspect, the bioreactor, method and system for cultivating microorganisms of the invention may be used for the production of biofuels, animal feed, waste water remediation, the production of high value compounds (e.g., oils), CO₂ remediation and/or the production of fertilizer or a combination thereof.

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Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of the common general knowledge in the field.

As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and

"comprised", are not intended to exclude further additives, components, integers or steps.

BRIEF DESCRIPTION OF THE DRAWINGS

To assist in understanding the invention and to enable a person skilled in the art to put the invention into practical effect, preferred embodiments of the invention will be described by way of example only with reference to the accompanying drawings, wherein:

FIG. 1 shows a perspective view of the bioreactor according to an embodiment.

10 FIG. 2 shows a cross section view of a portion of the bioreactor according to an embodiment.

FIG. 3 shows a cross-sectional view of the bioreactor, according to an embodiment.

FIG. 4 shows a cross-sectional view of the bioreactor according to an embodiment.

FIG. 5 shows a perspective view of the bioreactor according to two different embodiments.

FIG. 6 shows both a side view and top view of six alternate embodiments of the bioreactor (labeled I-VI).

FIG. 7 shows a perspective view of the bioreactor as shown in embodiment V of FIG. 6.

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FIG. 8 shows an example of a bioreactor arrangement as shown in embodiment IV of FIG. 6.

FIG. 9 shows a dense biofilm of algae growing on a first surface of a vertical liquid permeable layer.

FIG. 10 shows the harvesting process of three different microalgal cultivation systems, including an algal culture grown on a liquid permeable layer; a conventional laboratory algal liquid suspension culture; and a further algal

liquid suspension culture sourced from a different location, as discussed in the Examples.

DETAILED DESCRIPTION OF THE INVENTION

In one general embodiment, the present invention resides in a method and/or system for cultivating microorganisms, preferably photosynthetic and other benthic microorganisms and a bioreactor comprising at least one liquid permeable layer, comprising a first surface and a second surface, wherein photosynthetic microorganisms are cultivatable on the first surface of the liquid permeable layer and the second surface is connectable in fluid communication with a fluid source, and wherein the liquid permeable layer(s) is rotatable.

The bioreactor may further comprise a rotation member, the rotation member operating to rotate the liquid permeable layer(s).

- As shown in FIG. 1, which is a perspective view of a bioreactor 100 according to one embodiment, bioreactor 100 comprises a liquid permeable layer 110 comprising a first surface 111 and a second surface 112, which in the embodiment shown in FIG. 1, the liquid permeable layer 110 is cylindrical and in a vertical orientation. As further shown in FIG. 1, the first surface 111 of the liquid permeable layer 110 is shown to cultivate photosynthetic microorganisms 150, such as algae inclusive of micro algae. The bioreactor 100 of FIG. 1 is mounted on a stand 140 with a rotation member 130, which when in use, in the embodiment shown facilitates the rotation of the liquid permeable layer 110 vertically about a central axis 180. The liquid permeable layer 110 of the bioreactor 100 of FIG. 1 is supplied with water and nutrients from a fluid reservoir 160.
- Liquid permeable layer 110 may be constructed from any materials, either naturally porous or engineered to be porous, and/or perforated, for allowing fluid to permeate and facilitating adherence of the photosynthetic microorganisms 150 to be cultivated. The bioreactor 100 shown in FIG. 1, in one embodiment is situated in a housing 200, which allows light to penetrate through to the culturing photosynthetic microorganisms 150.

FIG. 2 shows a cross section view of a portion of a bioreactor. The liquid permeable layer 110 is shown to comprise a first surface 111 upon which photosynthetic microorganisms 150 are cultivated as a biofilm 151 and a second surface 112. The second surface 112 of the liquid permeable layer 110 is shown to be in fluid communication with a fluid source 120. In one embodiment, the fluid source 120 comprises a fluid conducting layer 121. It will be appreciated that fluid conducting material may be used to provide fluid and nutrients to the photosynthetic microorganisms 150. In the embodiment shown in FIG. 2, when in use, the photosynthetic microorganisms 150 are constantly supplied with water and nutrients *via* the liquid permeable layer 110 while also being provided with light and CO₂.

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FIG. 3 shows a cross sectional view of one embodiment of the bioreactor 100. The inventors as described in the examples, cultivated photosynthetic microorganisms on two static liquid permeable layers either in full light or limited 15 light to determine whether the level of light had an effect on the level of biomass produced. The liquid permeable layers were static during the experiments, although are capable of being rotated. In the embodiment shown in FIG. 3, the bioreactor 100 may be static as described above or may be rotated. The bioreactor 100, is arranged vertically on a stand 140, and comprises two liquid permeable 20 layers 110, between which a fluid source 120 is located and wherein the liquid permeable layers 110 comprise a first surface 111 wherein photosynthetic microorganisms 150 are cultured to form a biofilm 151 and a second surface 112 in fluid communication with the fluid source 120. In this embodiment, the same fluid source 120 supplies fluid and nutrients to both liquid permeable layers 110 25 and the fluid source is shown to comprise a fluid conducting layer 121. The fluid source can be seen in FIG. 3 to be supplied with water and nutrients from a fluid reservoir 160, wherein, when in use, the water and nutrients are pumped via pump 170 to the fluid conducting layer 121. Although a separate fluid source 120 for each liquid permeable layer 110 is also contemplated. It will also be appreciated 30 that varying the growth conditions of photosynthetic microorganisms 150, and in particular algae, will vary the rate of culturing and the final biomass

concentration. As further shown in FIG. 3, photosynthetic microorganisms 150 provided with limited light grown on a liquid permeable layer 110 of the bioreactor 100 results in a reduced culturing rate and a reduced overall biomass concentration when compared with photosynthetic microorganisms 150 provided with full light grown on a liquid permeable layer 110 of the bioreactor 100. In one embodiment, the light source 190 as shown in FIG. 3 may be natural light (e.g., the sun) or may be provided by an artificial source.

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FIG. 4 shows a cross section view of a bioreactor 100, in use, wherein the photosynthetic microorganisms 150, are shown as a uniform layer cultured on the 10 first surface 111 of liquid permeable layer 110, wherein the liquid permeable layer 110 has been configured as a cylinder and mounted on a stand 140 in a vertical orientation. The stand 140 comprises a rotation member 130 which in the embodiment shown in FIG. 4 is releasably coupled or connected to a motor 210 which facilitates rotation. The stand 140 upon which the bioreactor 100 shown in 15 FIG. 4 is positioned, is constructed such that the substantially cylindrical liquid permeable layer 110 rotates about a central axis 180. The second surface 112 of the liquid permeable layer 110 as shown in FIG. 4 is in fluid communication with a fluid source 120 which is shown to comprise a fluid conducting layer 121. The fluid conducting layer 121 can be seen in FIG. 4 to be supplied with water and 20 nutrients from a fluid reservoir 160, wherein, when in use, the water and nutrients are pumped via a pump 170 to the fluid conducting layer 120. When in use, the bioreactor 100 shown in FIG. 4 is rotated about a central axis 180 and the first surface 111 of the liquid permeable layer 110 upon which the photosynthetic microorganisms 150 are cultured is exposed to full light and limited light 25 intermittently as shown by the light sources 190.

FIG. 5 shows a perspective view of the bioreactor 100 according to two different embodiments. It will be appreciated that the positioning of the liquid permeable layer 110, upon which the photosynthetic microorganisms 150 are cultured relative to the light source 190 and/or any further required growth conditions may be varied to enhance the culturing of the specific photosynthetic microorganisms 150. In the embodiment shown in FIG. 5A, the liquid permeable

layer 110 is cylindrical and mounted on a stand 140 in a vertical orientation. The stand 140 comprises a rotation member 130 which in the embodiment shown in FIG. 5 is releasably coupled or connected to a motor 210 which facilitates rotation. The stand 140 upon which the bioreactor 100 shown in FIG. 5 is 5 positioned, is constructed such that the cylindrical liquid permeable layer 110, when in use, rotates about a central axis 180, wherein the light source 190 emits light from above the bioreactor 100. The liquid permeable layer 110 in FIG. 5A is in fluid communication with a fluid source. The fluid source in FIG. 5A is supplied with water and nutrients from a fluid reservoir 160, wherein, when in 10 use, the water and nutrients are pumped *via* a pump 170 to the fluid source. In the embodiment shown in FIG. 5B, the cylindrical liquid permeable layer 110 is positioned at an angle to the light source 190 to ensure the greatest exposure of the photosynthetic microorganisms 150 cultured on the liquid permeable later 110 to the light source 190. It will be appreciated that in some embodiments, an intermittent source of light may be preferred. As in FIG. 5B, the liquid permeable 15 layer 110 is in fluid communication with a fluid source. The fluid source in FIG. 5B, is supplied with water and nutrients from a fluid reservoir 160, wherein, when in use, the water and nutrients are pumped *via* a pump 170 to the liquid permeable layer 110. As shown in FIG. 5A and 5B, the bioreactor 100 is situated in a 20 housing 200, which allows light to penetrate through to the cultivating photosynthetic microorganisms 150.

FIG. 6 shows both a side view and top view of six alternate embodiments of the bioreactor 100 (labeled I-VI), wherein the liquid permeable layer 110 is configured to comprise different structures arranged around a central axis 180 and when in use, rotated about that axis 180. Embodiment I of FIG.6 comprises at least one liquid permeable layer 110 as a panel, wherein, either one or both sides of the panel comprise a first surface of the liquid permeable layer 110 for culturing photosynthetic microorganisms. Embodiment II of FIG. 6 comprises one liquid permeable layer 110 as a cylinder. Embodiment III of FIG. 6 comprises at least one liquid permeable layer 110 as an octagon. Embodiment IV of FIG. 6 comprises at least one liquid permeable layer 110 as two panels forming

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a cross. Embodiment V of FIG.6 comprises at least one liquid permeable layer 110 as three intersecting panels forming a stellate structure and embodiment VI of FIG. 6 comprises at least one liquid permeable layer 110 configured as four intersecting panels forming a stellate structure. Each of the six different embodiments in use, would be rotated around a central axis 180 to ensure maximum culturing conditions.

FIG. 7 shows a perspective view of an embodiment of the bioreactor 100 as shown in embodiment V of FIG. 6. In the embodiment shown in FIG. 7, there are two liquid permeable layers 110, comprising a first surface 111 wherein 10 photosynthetic microorganisms 150 are cultured to form a biofilm 151 and a second surface 112 in fluid communication with a fluid source 120 which is shown to comprise a fluid conducting layer 121 provided on each separately spaced panel 220. In this embodiment, a separate fluid conducting layer 121 supplies fluid and nutrients to the liquid permeable layers 110. Although the same 15 fluid conducting layer 121 for each liquid permeable layer 110 is also contemplated. When in use, the bioreactor 100 of the embodiment shown in FIG. 7 is rotated about a central axis 180 via rotation member 130, which in this particular embodiment is releasably coupled or connected to a motor 210 which facilitates rotation. Fluid source 120 is supplied with water and nutrients via a 20 pump 170 from fluid reservoir 160 and situated on stand 140.

FIG. 8 shows an example of a bioreactor 100 as shown in embodiment IV of FIG. 6, which rotates about a central axis 180, *via* rotation member 130, which in this particular embodiment is releasably coupled or connected to a motor 210, which facilitates rotation and acts as a stand 140.

FIG. 9 shows a dense biofilm of photosynthetic microorganisms 150, specifically a proprietary mix of freshwater algae, growing on a first surface 111 of a vertical liquid permeable layer 110, as cultivated in the experiments undertaken by the inventors.

EXAMPLES

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Example 1 is a non-limiting example of a bioreactor.

Proprietary mixed freshwater algae culture was successfully grown on two vertical 50cm x 13cm liquid permeable layers (FIG. 10), A and B, constantly supplied with nutrients and water from a fluid source through the liquid permeable layers. For the initial experiment, the vertical liquid permeable layers (A and B) did not rotate. The first surface of liquid permeable layer A was exposed to artificial light 12 hours a day and the first surface of liquid permeable layer B was shaded. A conventional suspended culture in a 2 Litre Schott-bottle was grown in parallel to allow for direct comparison.

In this instance the liquid permeable layers were formed from unprinted newspaper and the fluid source was fibre capillary matting fed with water and nutrients from a fluid reservoir. The algae culture was cultivated for 28 days, using L1 medium. Nitrate and phosphate consumption were monitored daily and both nutrients replenished as necessary. In addition, the culture medium for the algae biofilm culture was completely replaced after 7 and 14 days.

The biofilm culture grown on liquid permeable layer A grew the most during the experiment on the top half of the first surface and less well on the bottom half and on the first side of liquid permeable layer B, due to shading.

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At the end of cultivating the algae biofilm, both biofilms from the first and second liquid permeable layers A and B, in full light and shade were harvested and analysed for ash-free biomass, Fatty Acid Methyl Ester content (FAME) profile and carbohydrate content. The biofilms from liquid permeable layers A and B were harvested by scraping the algae off the liquid permeable layers with pre-weighted glass microscope slides.

Ash-free dry weight was measured directly on the slides and for biochemical profiling, the biofilm was washed off the slides with deionising water. The suspended algal culture was harvested by centrifugation of 40 ml of culture in a laboratory centrifuge and then transferred into glass beakers for dry weight determination. For biochemical profiling, 400 ml of culture was centrifuged and collected in several steps. Both cultures were then freeze-dried and analyzed.

It was immediately apparent during harvesting that the biofilm harvested from liquid permeable layers A and B was much denser than the suspended culture tested. This was confirmed by the dry weight measurements (Table 1, FIG. 10). On each side, 240 cm² (8 cm x 30 cm) of biofilm were harvested for dry weight, yielding a wet biomass of 2.3596 g for the light exposed side of liquid permeable layer A and 0.0994 g for the shaded side of liquid permeable layer B. After drying, this resulted in 0.2996 g and 0.0177 g of dried biomass, respectively. Adjusted for the whole cultivation surface (50 cm x 13 cm), this equalled 0.9783 g biomass on the light exposed side of liquid permeable layer A and 0.0420 g on the shaded side of liquid permeable layer B, for a total of 0.9783 g biomass from the biofilm. For comparison, the suspended control culture yielded 0.9600 g of dried biomass from 2 litres.

From the dry weight measurements, the biomass density/total solid content of the cultures could be calculated: 13.60% for the light exposed first surface of liquid permeable layer A, 14.49% for the shaded first surface of liquid permeable layer B and 0.048% for the suspended culture. The biofilm was 283 and 302 times more concentrated, depending on the cultivation side. On average, adjusted for total biomass produced on each surface, the biofilm has a total solid content of 13.64% equivalent to a concentration factor of 284 compared to the suspended culture.

For comparison, the same calculations were performed for an exemplary culture from a site in Townsville, Australia. Approximately 10'000 litres of culture were harvested with an Evodos centrifuge, yielding ca. 15000 g of wet algal paste. The paste was freeze-dried for ca 1900 g of dried biomass. The results in a biomass density/solid content of 0.019% for the original culture and a solid content of 13% for the algal paste coming out of the centrifuge, which was marginally lower than the solid content of the biofilm in the pilot experiment.

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The biochemical analysis showed that the biofilm had a higher lipid content than the suspended culture: 21.09% for the biofilm cultured on the light exposed first surface of liquid permeable layer A and 19.54% for the biofilm cultured on the shaded first surface of liquid permeable layer B, compared to

12.54% for the suspended culture. FAME content and carbohydrate content was only measured for the biofilm on the light exposed first surface of liquid permeable layer A and the suspended culture, as there was not enough biomass on the shaded first surface of liquid permeable layer B. FAME content was 9.45% (44.9% of total lipid) for the biofilm vs. 5.30% (42.3% of total lipid) for the suspended culture. Carbohydrate content was 46.01% for the biofilm versus 41.96% for the suspended culture.

In summary, it was demonstrated that the biomass productivity and quality were equal to or better than that of a suspended algae culture under the same conditions. However, as also demonstrated, the biomass was about 300 times more concentrated during cultivation on a liquid permeable layer and could be easily harvested by scraping the algae off the surface. As determined, productivity clearly differed when the algae growing on the first surface of the liquid permeable layer was provided with differing amounts of light.

As will be appreciated from the foregoing, the present invention provides an improved bioreactor, method and/or system for culturing microorganisms, preferably photosynthetic microorganisms. The bioreactor, method and system disclosed herein advantageously exhibits increased and uniform microorganism growth and productivity for generating high density cultivated biomass,
 specifically photosynthetic microorganisms, such as algae, as well as reduced contamination of the microorganisms and reduced harvesting time.

It will be apparent to persons skilled in the art that many modifications and variations may be made to the embodiments described without departing from the spirit or scope of the invention.

Each of the embodiments described herein may be used alone or in combination with one or more other embodiments of a bioreactor, method and system.

Throughout the specification, the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Various changes and modifications

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may be made to the embodiments described and illustrated without departing from the present invention.

	Vertical Area [m2]	Culture harvested after 28 days [g]	Dry biomass in harvested culture [g]	Biomass content of harvested culture [%]	Water content of harvested culture [%]	Concentration factor	Lipid content [%]	FAME content [%]	Carbohydrate content [%]
Front (Light Exposed)	0.024	6.882	0.936	13.60	86.40	283	21.09	9.459	46.01
Back (Shaded)	0.024	0.290	0.042	14.49	85.51	302	19.54	Not Measured	Not Measured
Total	0.048	7.172	0.978	13.63 ^b	86.36 ^b	284 ^b	21.02 ^b	Not Measured	Not Measured
2 Litre Suspended Culture	0.020 ^a	2000 ml	0.960	0.0422	99.96		12.540	5.301	41.960

 Table 1: Productivity of a non-rotating, bioreactor compared to a suspended culture

a: Based on the vertical cross-section of a 2 L Schott bottle.

b: Average weighted by dry biomass for each side.

c:	Not	sufficient	biomass	for	this	assay.

CLAIMS:

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1. A bioreactor for cultivating microorganisms, comprising;

at least one liquid permeable layer comprising a first surface and a second surface;

wherein microorganisms are cultivatable on the first surface of the liquid permeable layer and the second surface is connectable in fluid communication with a fluid source; and

wherein the liquid permeable layer is rotatable.

The bioreactor of claim 1, further comprising a rotation member for
 rotating the liquid permeable layer.

3. The bioreactor of claim 2, wherein the rotation member is automated and/or manually operated.

4. The bioreactor of any one of claims 1 to 3, wherein the liquid permeable layer is rotatable continuously and/or intermittently.

15 5. The bioreactor of any one of claims 1 to 4, wherein the liquid permeable layer is rotatable about a central axis of the liquid permeable layer or the bioreactor.

6. The bioreactor of any one of claims 1 to 5, wherein the fluid source

comprises a fluid conducting layer.

20 7. The bioreactor of claim 6, wherein the fluid conducting layer is in fluid communication with the second surface of the liquid permeable layer.

8. The bioreactor of claim 6 or claim 7, wherein the fluid conducting layer comprises a fluid conducting material.

9. The bioreactor of claim 8, wherein the fluid conducting material is
selected from the group consisting of: fabric, foam, glass fibres, synthetic polymers, natural fibres and printed materials.

10. The bioreactor of claim 9, wherein the fluid conducting material comprises capillary matting.

	11. The bioreactor of any one of claims 1 to 10, wherein the fluid source is selected from the group consisting of: water, nutrients, growth media, waste water and secondary treated sewage.
	12. The bioreactor of any one of claims 1 to 11, further comprising a fluid
5	reservoir.
	13. The bioreactor of any one of claims 1 to 12, further comprising a light
	source.
	14. The bioreactor of claim 13, wherein the light source is provided by natural light.
10	15. The bioreactor of claim 13, wherein the light source is provided by an artificial light source.
	16. The bioreactor of claim 15, wherein a substantial portion of the first surface of the liquid permeable layer is exposed to light.
15	17. The bioreactor of any one of claims 13 to 16, wherein the light source provides light intermittently.
	18. The bioreactor of any one of claims 13 to 17, wherein the light source provides light continuously.
	19. The bioreactor of any one of claims 1 to 18, further comprising a housing.
20	20. The bioreactor of claim 19, wherein the housing is transparent.21. The bioreactor of any one of claims 1 to 20, wherein the microorganisms are photosynthetic microorganisms, methanotrophic microorganisms, yeast, benthic microorganisms and combinations thereof.
	22. The bioreactor of claim 21, wherein the microorganisms are photosynthetic microorganisms.
25	23. The bioreactor of claim 22, wherein the photosynthetic microorganisms are algae, microalgae and/or cyanobacteria.
	24. The bioreactor of any one of claims 1 to 23, further comprising a gas source.

25. A method for cultivating microorganisms, the method comprising:

rotating at least one liquid permeable layer comprising a first surface and a second surface, wherein the second surface is in fluid communication with a fluid source; and

cultivating the microorganisms on the first surface.

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26. The method of claim 25, wherein at least one liquid permeable layer is rotated by a rotation member.

27. The method of claim 26, wherein the rotation member is automated and/or manually operated.

10 28. The method of any one of claims 25 to 27, wherein the liquid permeable layer is rotated continuously and/or intermittently.

29. The method of any one of claims 26 to 28, wherein the rotation member rotates a platform comprising the liquid permeable layer.

30. The method of any one of claims 25 to 29, wherein the liquid permeable layer is rotated about a central axis of the liquid permeable layer.

31. The method of any one of claims 25 to 30, wherein the fluid source comprises a fluid conducting layer.

32. The method of claim 31, wherein the fluid conducting layer comprises a fluid conducting material.

20 33. The method of claim 32, wherein the fluid conducting material is selected from the group consisting of: fabric, foam, glass fibres, synthetic polymers, natural fibres and printed materials.

34. The method of claim 33, wherein the fluid conducting material comprises capillary matting.

25 35. The method of any one of claims 25 to 34, wherein the fluid source is selected from the group consisting of: water, nutrients, growth media, waste water and secondary treated sewage.

36. The method of any one of claims 25 to 35, wherein the fluid source is supplied by a fluid reservoir.

37. The method of any one of claims 25 to 36, wherein the liquid permeable layer is provided with a light source.

38. The method of claim 37, wherein the light source is natural light.

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39. The method of claim 37, wherein the light source is an artificial light source.

40. The method of any one of claims 37 to 39, wherein a substantial portion of the first surface of the liquid permeable layer is exposed to the light source.

10 41. The method of any one of claims 25 to 40, wherein the microorganisms are photosynthetic microorganisms, methanotrophic microorganisms, yeast, benthic microorganisms and combinations thereof.

42. The method of claim 41, wherein the microorganisms are photosynthetic microorganisms.

15 43. The method of claim 42, wherein the photosynthetic microorganisms are algae, micro-algae and/or cyanobacteria.

44. The method of any one of claims 25 to 43, wherein the liquid permeable layer is provided with one or more gases.

45. A system for cultivating microorganisms, the system comprising:

20 at least one liquid permeable layer comprising a first surface comprising microorganisms and a second surface connected in fluid communication with a fluid source; and

a rotation member for rotating the liquid permeable layer, to facilitate microorganism growth.

25 46. The system of claim 45, wherein the rotation member is automated and/or manually operated.

47. The system of claim 45 or claim 46, wherein the liquid permeable layer is rotated continuously and/or intermittently.

48. The system of any one of claims 45 to 47, wherein the rotation member rotates a platform comprising the liquid permeable layer.

49. The method of any one of claims 45 to 48, wherein the liquid permeable layer is rotated about a central axis of the liquid permeable layer.

50. The system of any one of claims 45 to 49, wherein the fluid source comprises a fluid conducting layer.

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51. The system of claim 50, wherein the fluid conducting layer comprises a fluid conducting material.

52. The system of claim 51, wherein the fluid conducting material is selected
from the group consisting of: fabric, foam, glass fibres, synthetic polymers, natural fibres and printed materials.

53. The system of claim 52, wherein the fluid conducting material comprises capillary matting.

54. The system of any one of claims 45 to 53, wherein the fluid source is
selected from the group consisting of: water, nutrients, growth media, waste water and secondary treated sewage.

55. The system of any one of claims 45 to 54, further comprising a fluid reservoir which provides the fluid source.

56. The system of any one of claims 45 to 55, further comprising a light source to facilitate microorganism growth.

57. The system of claim 56, wherein the light source is natural light.

58. The system of claim 56, wherein the light source is an artificial light source.

59. The system of any one of claims 56 to 58, wherein a substantial portion of the first surface of the liquid permeable layer is exposed to the light source.

60. The system of any one of claims 45 to 59, wherein the microorganisms are photosynthetic microorganisms, methanotrophic microorganisms, yeast, benthic microorganisms and combinations thereof.

61. The system of claim 60, wherein the photosynthetic microorganisms are algae, microalgae and/or cyanobacteria.

62. The system of any one of claims 45 to 61, further comprising a gas source for the supply of one or more gases to the liquid permeable layer, to facilitate microorganism growth.



1 / 10

FIGURE 1



FIGURE 2









FIGURE 4



FIGURE 5


FIGURE 6

7/10



FIGURE 7





9/10

FIGURE 9



Appendix 5.1 Variables for simulation

Naming conventions

Variables are named to indicate the type of metric they represent with the initial normal sized letters and the specific system components this metric applies to with the subscript (Table 1).

METRIC_{INDICATOR}

The last three letter of the descriptor can indicate that a metric is only used in a certain system. $_{BAG}$ refers to metrics used in the batch bag process, $_{SCB}$ refers to metrics used in the semi-continuous suspension bags, $_{PAN}$ refers to the batch cultivation of biofilms on panels and $_{SCP}$ refers to semi-continuous cultivation of biofilms on panels. For metrics that are the same regardless of system, the last three letters are omitted.

Table 1: Variable names used in the simulation. Variables are presented in the order of appearance, grouped per system. Variables that have appeared before with a different indicator are only repeated if the dimensions or values have changed.

Baseline: Batch suspension system			
Variable	Value	Dimension	Description
N _{BAG}	20		Number of bags
V _{BAG}	500	1	Volume of each bag
X _{INOC}	0.6	g ⁻¹	Biomass concentration of inoculation culture
X _{OBAG}	0.0625	g ⁻¹	Starting biomass concentration for each bag
VINOCBAG		I	Volume of inoculation culture
P _{INOC}		KWh l ⁻¹	Power consumed for the preparation of the inoculum
P _{FILLBAG}		KWh l ⁻¹	Power consumed for filling the bags
μ1	0.3231	d ⁻¹	Specific growth rate day 0 - 7
μ ₂	0.0532	d ⁻¹	Specific growth rate day 7-28
X _t		g ⁻¹	Biomass concentration at time t
Variable	Value	Dimension	Description

P _{AERBAG}	0.0026	$KWh l^{-1} d^{-1}$	Power used for aeration	
T _{LAMPBAG}	05		Duration of illumination	
N _{LAMPBAG}	14		Number of lamps per bag	
P _{LAMP}	1.008	KWh d⁻¹	Power consumed by each lamp	
Тватснвад	1-28	d	Duration of the batch phase	
B _{HARVBAG}		g	Overall biomass	
V _{HARVBAG}		I	Volume harvested	
X _{TBATCHBAG}		g ⁻¹	Biomass concentration at the time of harvesting	
P _{SEPBAG}	1.2 * 10 ⁻³	KWh l⁻¹	Power consumption for the separation	
P _{DISCBAG}	2 * 10 ⁻⁵	KWh g ⁻¹	Power consumption for the discharge	
T _{reset}	2	d	Duration to clean and reset system	
T _{TOT}	365	d	Total duration modelled	
YB _{BAG}		kg yr⁻¹	Yearly biomass productivity	
YP _{BAG}		KWh yr⁻¹.	Yearly power consumption	
T _{CYCBAG}		d	Duration of cultivation cycle	
N _{CYCBAG}		yr ⁻¹	Number of cycles per year	
SP _{BAG}		KWh kg⁻¹	Specific power consumption	
Scenario 1: Semi-continuous suspension process				
The indicator ' _{BAG} ' is replaced by ' _{SCB} ', where appropriate, eg. N_{CYCBAG} becomes N_{CYCSCB} and YB_{BAG} becomes YB_{SCB} .				

Variable	Value	Dimension	Description
T _{RGSCB}	1-T _{BATCHSCB}	d	Duration of regrowth
X _{TBATCHSCB}		g ⁻¹	Biomass concentration at the end of the batch phase
X _{TBATCHSCB} -TRGSCB		g ⁻¹	Target biomass concentration for regrowth

Scenario 2: Biofilm batch cultivation

VP_{BAG}

 AP_{PAN}

The indicator '___PAN' is used. The process is simulated for both materials, with parameters specific to one material being distinguished the subscripts "FG or "PVDF. E.g. the number of FG panels needed would be N_{PANFG} .

Variable	Value	Dimension	Description			
A _{PAN}	2	m²	Area of each panel			
N _{PAN}			Number of panels			
X _{0PANFG}	18.84	g m ⁻² Starting biomass concentr g m ⁻² each panel	Starting biomass concentration for			
X _{0PANPVDF}	7.31		each panel			
P _{VACPAN}	1.0*10 ⁻⁴	KWh l⁻¹	Power for vacuum filtration			
μ1	0	d ⁻¹	Specific growth rate day 0 – 3 (FG)			
	0		Specific growth rate day 0 – 3 (PVDF)			
μ ₂	0.0666	d ⁻¹	Specific growth rate day 3 – 10 (FG)			
	0.1428		Specific growth rate day 3 – 10 (PVDF)			
μ ₃	0.0161	d ⁻¹	Specific growth rate day 10 – 28 (FG)			
	0.0211		Specific growth rate day 10 – 28 (PVDF)			
A _{HARVPAN}	2	m ²	Area harvested per panel			
Scenario 3: Biofilm semi-continuous cultivation						
The indicator ' _{SCP} ', is used, in conjunction with $_{FG}$ or $_{PVDF}$ where appropriate, eg. $X_{OPANPVDF}$ becomes $X_{OSCPPVDF}$.						
Evaluation						
Variable	Value	Dimension	Description			
VP _{BAG}		g l ⁻¹ d ⁻¹	Volumetric productivity			

Areal productivity

g m⁻² d⁻¹

Appendix 5.2: Microalgal growth methods

Microalgal cultivation:

Based on experimental of suspension and biofilm cultivation at laboratory scale, as part of a Special Topic Project (Zanoni 2014) at James Cook University. All measurements were determined as the average of three independent samples.

Inoculation cultures for the Prymnesiophyte *Isochrysis aff. galbana* (TISO, NQAIF 001) were prepared by growing TISO for 7 days in f/2 (Guillard and Ryther 1962; Guillard 1975), under 56.7 μ m of fluorescent light (Phillips 36W TLD) with a 12:12h photoperiod and constant aeration with ambient air

Suspension cultures were set-up in 1000 ml aerated bottles with the same medium, inoculated at 0.625 g l^{-1} biomass concentration and cultivated the same conditions as above, for 28 days.

Biofilm cultures grown on two different types of 47 mm vacuum filters, made from Fibre glass (FG A, Micro Science) or Polyvinylidene fluoride (PVDF 0.45 μ m pore size, Millipore), respectively. The filters were inoculated by vacuum filtering sufficient inoculation culture (as above) to achieve a starting biomass concentration of 18.84 g m⁻² for FG and 7.31 g m⁻² for PVDF. The inoculated filters were placed on in 50 mm petri-dishes, on agar plates consisting of f/2 (Guillard and Ryther 1962; Guillard 1975) solidified with agar (20 g l⁻¹). The petri-dishes were placed in small plastic greenhouses (27cm x 35cm), under the same light conditions as the suspension cultures. Humidity was controlled by spraying the inside of the greenhouses with distilled water once per day and by placing beakers with water in the greenhouses. Biofilms were randomly sampled, based on computer generated random numbers (MS Excel).

Biomass determination:

Suspension biomass was determined gravimetrically by centrifuging 40 ml of suspension biomass in at 4000rpm (Eppendorf 5810R). The supernatants were carefully aspirated, and the pellets were resuspended in 4.5mL of distilled water and

175

transferred into tared 10mL beakers (Schott). Cultures were dried at 105 °C in a Milestone – Pyro High Temperature Microwave Muffle Furnace for 3h 40 min.

Biofilm biomass was determined gravimetrically, by pre-drying labeled filters at 105 °C in a Milestone – Pyro High Temperature Microwave Muffle Furnace for 3h 40 min. The filters were pre-weighing (Tare weight) before inoculation. For sampling, the biofilms were again dried at 105 °C in a Milestone – Pyro High Temperature Microwave Muffle Furnace and weighted to determine the difference in biomass.

Appendix 5.3: Power use

Electricity cost is modelled by estimating a power cost (in KWh) to every processing step (Figure), based on literature values (where available) and standard equations. Cultivation efficiency is calculated by dividing the total power consumption by the amount of biomass



Figure 1: Power costs occurring during the production process

produced.

Inoculation step:

Preparation of inoculum culture (PINOCULUM KWh l-1):

Based on the light and aeration requirements for 7 days of suspension cultivation in bags (see below). No additional inoculum power (done manually and to avoid recursion) and harvesting cost (not needed), calculated per litre of inoculum.

$$P_{INOC} = \left(\frac{(N_{LAMPBAG} * P_{LAMPBAG} * T_{LAMPBAG})}{V_{BAG}} + P_{AERBAG}\right) * 7 \ days$$
(Eq 1)

Filling of bags (P_{FILL}, KWh l⁻¹)

Power required to fill the bags was calculated as the pumping energy (REF) required for filling a 1.5 m tall bag with 500 l in 5 minutes.

$$P_{FILL} = \frac{q * \rho * g * h}{3.6 * 10^6 * \eta} * t$$
 (Eq 2)

With the flow rate (q m³ h⁻¹), density of water (ρ [kg m⁻³], gravity (g, 9.81 m s⁻²), hydraulic lift (h, [m]) and pump efficiency (η []).

Vacuum filtration (PVACUUM): Biofilm only

Molina Grima et al. (2003) provides data for filtration of with vacuum suction filters. Based on this source, power consumption is 0.1 KWh per m³ of solution.

Cultivation

Number of lamps (NLAMP): Both systems

Based on the lights used in NQAIF with provided the illumination used for Nicole's data. This set-up used panels of 7 fluorescent tubes (Phillips 36W/840) that were able to light up a space of ca. 70 cm deep and ca. 1.5 m long (1.05 m²), on which the biofilms and suspension cultures were grown. This is equivalent to 7 lamps per m² of Illuminated surface, which is used for biofilms. The radius of a cylindrical 500 l big bag can be calculated:

$$r = \sqrt{\frac{V}{h * \pi}}$$
(Eq. 3)

With the volume V [I] and the fill height h [m]. From this it is possible to calculate the mantle area (M, [m²]):

$$M = 2 * \pi * r * h \tag{Eq. 4}$$

Which results in 2.05 m^2 illuminated area per bag - close enough to 2 m^2 to justify a light panel on each side of the bag, for a total of 14 lamps per bag.

Power of lamps (PLAMP, TLAMP)

According to manufacturer documentations, the TL-D 36W/840 1PP has an energy consumption of 42 KWh per 1000 h or 1.008 kWh d⁻¹ per lamp. This is equal to 7.056 kWh per bag or panel per day (with a 12:12 on:off cycle).

Areation: Bag only

Sanchez Miron et al. (2000) gives specific power consumption of 0.109 kW m-3 for microalgal cultivation in a bubble column reactor, which is equivalent to 0.5232 KWh d-1 per bag. Compared to the power used for light, this is a fairly small cost.

Recircultation: Panel only

For recirculation, a panel recirculates 5 l h-1 (based experiences from my panel experiments and in line with Naumann et al. (2012)systems). Using EQ 2, with a Hydraulic lift of 1.2 m. This results in 0.4205 KWh per $m^2 d^{-1}$

Harvesting

Centrifugation: Suspension only

Power consumption is available from the manufacturer for the Evodos 25 centrifuge. The centrifuge consumes 1.2 KWh per m³ of suspension processed, plus 0.2 KWh per 10 kg of discharged compressed solids.

This corresponds with the information in (Molina Grima et al. 2003) who reports 0.9 - 8 KWh per m³ of culture for centrifugation systems that can be used for concentration to paste density.

Scraping: Panel system

There is no available data for an automated harvesting system for biofilm panels, current (research) systems are harvested manually.

Appendix 5.4: MATLAB code